

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12670 A1

- (51) International Patent Classification⁷: C07K 14/705, 14/715, C12N 5/10, 15/12, 15/63, 15/64
- (21) International Application Number: PCT/US00/21736
- (22) International Filing Date: 10 August 2000 (10.08.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/148,682 13 August 1999 (13.08.1999) US
60/154,887 20 September 1999 (20.09.1999) US
- (71) Applicant (*for all designated States except US*): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832 (US). NI, Jian [CN/US]; 5502 Manorfield Road, Rockville, MD 20853 (US).

(74) Agents: HOOVER, Kenley, K. et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12670 A1

(54) Title: TGF-BETA RECEPTOR POLYNUCLEOTIDES, POLYPEPTIDES, AND ANTIBODIES

(57) Abstract: The present invention relates to novel human TGF-beta receptor polypeptides and isolated nucleic acids containing the coding regions of the genes encoding such polypeptides. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human TGF-beta receptor polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human TGF-beta receptor polypeptides.

TGF-beta Receptor Polynucleotides, Polypeptides, and Antibodies

Field of the Invention

5 The present invention relates to novel members of the Transforming Growth Factor-beta (TGF-beta) receptor family of proteins. More specifically, isolated nucleic acid molecules are provided encoding novel TGF-beta receptor polypeptides. Novel TGF-beta receptor polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human
10 TGF-beta receptor polynucleotides and/or polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel TGF-beta receptor polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to
15 methods and/or compositions for inhibiting the production and function of the polypeptides of the present invention.

Background of the Invention

20 Members of the superfamily of secreted proteins known as the Transforming Growth Factor-beta (TGF-beta) family of proteins are cytokines that affect a wide range of biological functions, and includes the activin and *dpp/Vg*-related subfamilies of proteins (Kim, D., et al., *J. Biomed. Sci.*, 3:143-58 (1996)). TGF-beta is thought to inhibit the growth of multiple epithelial cell lines, and antisense inhibition of TGF-beta has resulted in increased
25 tumorigenicity of weakly tumorigenic cell lines (Markowitz, S. et al., *Science*, 268:1336-8

(1995)). Additional activities associated with members of the TGF-beta superfamily of proteins includes developmental regulation in vertebrates, hematopoiesis and immune and inflammatory responses, and the induction of extracellular matrix production.

TGF-beta proteins illicit biological responses through binding to specific cell-surface receptors composed of two related transmembrane serine/threonine kinases, named the Type I and Type II TGF-beta receptors, respectively (T β R-I and T β R-II) (Luo, K. et al., *EMBO J.*, 15:4485-96 (1996); Huse, M. et al., *Cell*, 96:425-36 (1999)). Both T β R-I and T β R-II receptors consist of the same domains, namely a cysteine-rich N-terminal extracellular domain involved in ligand binding, a transmembrane domain, and a C-terminal cytoplasmic kinase domain (Chen, F. et al., *Proc. Natl. Acad. Sci.*, 92:1565-9 (1995)). TGF-beta bound to the T β R-II receptor induced formation of heteromeric complexes between T β R-I and T β R-II resulting in phosphorylation of serine and threonine residues in the GS region of T β R-I (Wrana, J. et al., *Nature*, 370:341-6 (1994)). Phosphorylation of T β R-I by T β R-II activates the receptor kinase of T β R-I, which phosphorylates and activates transcription factors, such as TAK-1, the Smad proteins, or further members of the MADR family of signal transduction molecules (Wrana, J.L., *Miner Elect. Metab.*, 24:120-30 (1998)).

It was demonstrated by Markowitz et al. that colon cancer cell lines harboring mutations in the gene encoding T β R-II resulted in inactivation of the T β R-II receptor. Further, it was determined that a subset of colon cancer cells encoded for an inactive T β R-II receptor, allowing these cells to avoid TGF-beta-mediated growth inhibition (Markowitz, S. et al., *Science*, 268:1336-8 (1995); Kim, D., et al., *J. Biomed. Sci.*, 3:143-58 (1996)).

Likewise, Kadin, et al. found that the loss of responsiveness to TGF-beta by T-cell lymphoma cells correlated with a reduction in the expression of the T β R-II receptor (Kadin et al., *Proc. Natl. Acad. Sci. USA*, 91:6002-06 (1994)). Kim et al. determined that loss of sensitivity to TGF-beta by the prostate cancer cell line LNCaP was due to a genetic change in the T β R-I receptor gene in these cells. LNCaP cells transiently transfected with T β R-I cDNA

restored the sensitivity of these cells to TGF-beta (Kim, I. et al., *Cancer Res.*, 56:44-8 (1996)).

Based upon these and other studies, it is thought that a loss of expression of T β R-II and/or T β R-I, or the expression of inactive forms of T β R-II and/or T β R-I, may contribute to 5 the unregulated growth of lymphocytes in malignant lymphomas, as well as other cancerous cells. Loss of expression of T β R-I and/or T β R-II may serve as a prognostic marker indicative of the progression of some cancers.

Thus, there exists a clear need for identifying and exploiting novel members of the TGF-beta receptor family of proteins. Although structurally related, such proteins may 10 possess diverse and multifaceted functions in a variety of cell and tissue types. Receptor type molecules should prove useful in target based screens for small molecules and other such pharmacologically valuable factors. The genes encoding TGF-beta receptors of the present invention are also useful in gene therapy-based therapeutic applications, restoring the wild-type activity of TGF-beta receptors to cells expressing inactive TGF-beta receptors. 15 Additionally, assays designed to monitor the expression of T β R-I and/or T β R-II may be useful as diagnostic tools to monitor the presence or progression of certain types of cancers.

Summary of the Invention

The present invention includes isolated nucleic acid molecules comprising, or 20 alternatively, consisting of a polynucleotide sequence disclosed in the sequence listing and/or contained in a human cDNA plasmid described in Table 1 and deposited with the American Type Culture Collection (ATCC). Fragments, variants, and derivatives of these nucleic acid molecules are also encompassed by the invention. The present invention also includes isolated nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide 25 encoding TGF-beta receptor polypeptides. The present invention further includes TGF-beta receptor polypeptides encoded by these polynucleotides. Further provided for are amino acid

sequences comprising, or alternatively, consisting of, TGF-beta receptor polypeptides as disclosed in the sequence listing and/or encoded by the human cDNA plasmids described in Table 1 and deposited with the ATCC. Antibodies that bind these polypeptides are also encompassed by the invention. Polypeptide fragments, variants, and derivatives of these 5 amino acid sequences are also encompassed by the invention, as are polynucleotides encoding these polypeptides and antibodies that bind these polypeptides.

Detailed Description

Tables

10 Table 1 summarizes ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application. Table 1 further summarizes the information pertaining to each "Gene No." described below, including cDNA clone identifier, the type of vector contained in the cDNA clone identifier, the nucleotide sequence identifier number, nucleotides contained in the disclosed sequence, the location of 15 the 5' nucleotide of the start codon of the disclosed sequence, the amino acid sequence identifier number, and the last amino acid of the ORF encoded by the disclosed sequence.

Table 2 indicates public ESTs, of which at least one, two, three, four, five, ten, or more of any one or more of these public EST sequences are optionally excluded from certain embodiments of the invention.

20 Table 3 summarizes the expression profile of polynucleotides corresponding to the clones disclosed in Table 1. The first column provides a unique clone identifier, "Clone ID NO:Z", for a cDNA clone related to each contig sequence disclosed in Table 1. Column 2, "Library Code" shows the expression profile of tissue and/or cell line libraries which express the polynucleotides of the invention. Each Library Code in column 2 represents a tissue/cell 25 source identifier code corresponding to the Library Code and Library description provided in Table 5. Expression of these polynucleotides was not observed in the other tissues and/or cell

libraries tested. One of skill in the art could routinely use this information to identify tissues which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue expression.

5 Table 4, column 1, provides a nucleotide sequence identifier, "SEQ ID NO:X," that matches a nucleotide SEQ ID NO:X disclosed in Table 1, column 5. Table 4, column 2, provides the chromosomal location, "Cytologic Band or Chromosome," of polynucleotides corresponding to SEQ ID NO:X. Chromosomal location was determined by finding exact matches to EST and cDNA sequences contained in the NCBI (National Center for
10 Biotechnology Information) UniGene database. Given a presumptive chromosomal location, disease locus association was determined by comparison with the Morbid Map, derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine
15 (Bethesda, MD) 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>). If the putative chromosomal location of the Query overlapped with the chromosomal location of a Morbid Map entry, the OMIM reference identification number of the morbid map entry is provided in Table 4, column 3, labelled "OMIM ID." A key to the OMIM reference identification numbers is provided in Table 6.

20 Table 5, column 1, provides the Library Code disclosed in Table 3, column 2. Column 2 provides a description of the tissue or cell source from which the corresponding library was derived.

Table 6 provides a key to the OMIM reference identification numbers disclosed in Table 4, column 3. OMIM reference identification numbers (Column 1) were derived from
25 Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine,

(Bethesda, MD) 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>). Column 2 provides diseases associated with the cytologic band disclosed in Table 4, column 2, as determined from the Morbid Map database.

5 Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by 10 the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including 15 those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X (as described in column 5 of Table 1), or cDNA 20 plasmid:Z (as described in column 3 of Table 1 and contained within a pool of plasmids deposited with the ATCC). For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without a natural or artificial signal sequence, the protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. 25 Moreover, as used herein, a "polypeptide" refers to a molecule having an amino acid sequence encoded by a polynucleotide of the invention as broadly defined (obviously

excluding poly-Phenylalanine or poly-Lysine peptide sequences which result from translation of a polyA tail of a sequence corresponding to a cDNA).

In the present invention, a representative plasmid containing the sequence of SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC") and/or described 5 in Table 1. As shown in Table 1, each plasmid is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number (ATCC Deposit No:Z). Plasmids that were pooled and deposited as a single deposit have the same ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the 10 deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, or the complement thereof (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein) and/or sequences contained in 15 cDNA plasmid:Z (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein). "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μ g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C. 20

Also included within "polynucleotides" of the present invention are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower 25 percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a

solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH₂PO₄; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt 5 concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. 10 The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since 15 such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or 20 modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the 25 polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or

RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

5 In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a
10 portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

15 "SEQ ID NO:X" refers to a polynucleotide sequence described in column 5 of Table 1, while "SEQ ID NO:Y" refers to a polypeptide sequence described in column 10 of Table 1. SEQ ID NO:X is identified by an integer specified in column 6 of Table 1. The polypeptide sequence SEQ ID NO:Y is a translated open reading frame (ORF) encoded by polynucleotide SEQ ID NO:X. The polynucleotide sequences are shown in the sequence listing immediately
20 followed by all of the polypeptide sequences. Thus, a polypeptide sequence corresponding to polynucleotide sequence SEQ ID NO:2 is the first polypeptide sequence shown in the sequence listing. The second polypeptide sequence corresponds to the polynucleotide sequence shown as SEQ ID NO:3, and so on.

25 The polypeptides of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be

modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

(See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992)).

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced

polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature 5 form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, 10 and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using 15 techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length 20 (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

25 "A polypeptide having functional activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention,

including mature forms, as measured in a particular assay, such as, for example, a biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., 5 the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

The functional activity of the polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

10 For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the present invention for binding to an antibody to the full length polypeptide, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" 15 immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one 20 embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

25 In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can

be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., *Microbiol. Rev.* 59:94-123 (1995). In another embodiment, physiological correlates polypeptide of the present invention binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants derivatives and analogs thereof to elicit polypeptide related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and 10 are within the scope of the invention.

Polynucleotides and Polypeptides of the Invention

FEATURES OF PROTEIN ENCODED BY GENE NO: 1

15 Translation products corresponding to this gene share sequence homology with a human protein kinase (HPK) (See International Publication No. WO9811234), as well as with the murine Nek1 serine/threonine- and tyrosine-specific protein kinase (See Genbank Accession AAB23529), which is thought to be important in cell signaling and the regulation of cell differentiation and/or proliferation.

20 The gene encoding the translation product of the present invention is a member of the TGF-beta receptor family of proteins. Proteins belonging to this family contain a cytoplasmic serine/threonine kinase domain. In order to identify these kinase domains, the following consensus pattern has been developed (Swiss Institute of Bioinformatics) :

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3). Preferred polypeptides of 25 the invention comprise, or alternatively consist of, the following amino acid sequence: VMHRDIKPANVFI (SEQ ID NO: 26). Fragments and/or variants of these polypeptides,

such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides. Further preferred are polypeptides comprising the serine/threonine kinase domain of SEQ ID NO: 26, 5 and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of SEQ ID NO: 14. The additional contiguous amino acid residues may be N-terminal or C-terminal to the serine/threonine kinase domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the serine/threonine kinase domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified 10 number.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, or all nine of the immunogenic epitopes shown in SEQ ID NO: 14 as residues: Met-1 to Gln-9, Pro-14 to Leu-22, Glu-37 to Arg-42, Phe-126 to Pro-134, His-209 to Asp-218, Pro-235 to Met-241, Lys-249 to Asp-254, Pro-257 15 to Leu-267, and Pro-277 to Asp-284. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed primarily in liver hepatoma tissue, as well as in musculo- 20 skeletal system and smooth muscle tissues.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: liver hepatomas, as well as diseases and/or disorders of the musculo-skeletal 25 system, and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell

type(s). For a number of disorders of the above tissues or cells, particularly of the hepatic and musculo-skeletal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., liver, musculo-skeletal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in liver hepatoma tissue and musculo-skeletal tissues, and the homology to HPK and Nek1 proteins, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of liver hepatomas and cancers in general, as well as for diseases and/or disorders of the musculo-skeletal system.

The tissue distribution in liver hepatoma tissue indicates that polynucleotides, translation products and antibodies corresponding to this gene may be useful in the regulation of the proliferation of liver hepatomas, and may serve as a marker to indicate the presence or growth of liver hepatomas. Therefore, this gene is a good target for agonists, particularly antibodies, which promote receptor activities such as signal transduction and/or the inhibition of cellular proliferation, for example. Accordingly, preferred are antibodies which specifically bind a portion of the translation product of this gene. Also provided is a kit for detecting liver hepatomas. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting liver hepatomas in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The

above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein.

Alternatively, the tissue distribution in smooth muscle tissue indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions and pathologies of the cardiovascular system, such as heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis, and wound healing. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

10 FEATURES OF PROTEIN ENCODED BY GENE NO: 2

Translation products corresponding to this gene share sequence homology with a number of kinase proteins (See Genbank Accession CAA88531, for example), which are thought to be involved in signal transduction.

The gene encoding the translation product of the present invention is a member of the TGF-beta receptor family of proteins. Proteins belonging to this family contain a cytoplasmic serine/threonine kinase domain. In order to identify these kinase domains, the following consensus pattern has been developed:

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3). Preferred polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

VIHRDLKSRNVVI (SEQ ID NO: 27). Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides. Further preferred are polypeptides comprising the serine/threonine kinase domain of SEQ ID NO: 27, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of SEQ ID NO: 15. The additional contiguous amino acid residues may be N-terminal or C-terminal

to the serine/threonine kinase domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the serine/threonine kinase domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number.

5 Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or all twenty of the immunogenic epitopes shown in SEQ ID NO: 15 as residues: Asn-95 to Glu-101, Asp-246 to Phe-253, Met-263 to Ser-275, Arg-293 to Arg-299, Phe-303 to Arg-314, Glu-319 to Asn-327, Val-352 to 10 Ser-358, Ser-424 to Lys-434, Pro-448 to Lys-456, Tyr-516 to Lys-527, Ser-535 to Glu-542, Thr-555 to Asp-570, Thr-586 to Ser-593, Phe-605 to Ser-610, Asn-631 to Gln-640, Asn-653 to Gly-684, Ser-690 to Lys-713, Asn-723 to Asn-741, His-749 to Val-763, and Glu-772 to Trp-797. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides 15 encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed in bone marrow and osteoblasts, as well as in primary dendritic cells.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, 20 are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of the skeletal system and the immune system, and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). 25 For a number of disorders of the above tissues or cells, particularly of the skeletal and immune systems, expression of this gene at significantly higher or lower levels may be

routinely detected in certain tissues or cell types (e.g., skeletal, bone marrow, osteoblasts, immune, primary dendritic cells, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in bone marrow, primary dendritic cells, and osteoblasts, and the homology to a number of kinase proteins, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the immune and skeletal system. Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

Expression of this gene product in bone marrow and primary dendritic cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, expression of this gene product in osteoblasts suggests that it may play a role in the survival, proliferation, and/or growth of osteoblasts. Therefore, it may be useful in influencing bone mass in such conditions as osteoporosis. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation 5 products, may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 3

The translation product of this gene shares sequence homology with a number of 10 kinase proteins, such as for example, the human STE20-like stress activated serine/threonine kinase (See International Publication No. WO9915635), which is thought to be involved in such cellular processes as signal transduction. Based upon the homology it is believed that these proteins will share at least some biological activities.

Preferred polypeptides of the present invention comprise, or alternatively consist of, 15 one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or all twelve of the immunogenic epitopes shown in SEQ ID NO: 16 as residues: Ile-42 to Gln-47, Glu-58 to Glu-63, Cys-77 to Val-82, Ser-137 to Ile-145, Thr-172 to Thr-178, Ser-194 to Asp-201, Ala-213 to His-223, Leu-255 to Ala-265, Asn-277 to Tyr-283, Arg-290 to Trp-324, Thr-328 to Val-338, and Leu-364 to Gln-376. Fragments and/or variants of these polypeptides, such as, 20 for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed primarily in placental tissue, T-cells and T cell lymphoma.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, 25 are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not

limited to: diseases and/or disorders of the placenta, developing systems, and the immune system, particularly T cell lymphoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or 5 cells, particularly of the placenta, and the immune and developing systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, developing, placenta, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression 10 level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in placental tissue, T cell lymphoma and T-cells, and the homology to several kinase proteins thought to be involved in signal transduction and protein activation, indicates that polynucleotides, translation products and antibodies corresponding 15 to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the placenta, developing systems, and the immune system. Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

20 The tissue distribution suggests that the protein product of this clone is useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta suggests that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the 25 development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also suggests that this gene product may

be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body.

Likewise, expression of this gene product in T-cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 4

The translation product of this gene shares sequence homology with a number of kinase and serine/threonine kinase proteins (See Genbank Accessions CAA66229 or CAB06295, or International Publication No. WO9801756).

25 The gene encoding the translation product of the present invention is a member of the TGF-beta receptor family of proteins. Proteins belonging to this family contain a cytoplasmic

serine/threonine kinase domain. In order to identify these kinase domains, the following consensus pattern has been developed:

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3). Preferred polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

- 5 VVHRDLKLENILL (SEQ ID NO: 28). Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides. Further preferred are polypeptides comprising the serine/threonine kinase domain of SEQ ID NO: 28,
- 10 and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of SEQ ID NO: 17. The additional contiguous amino acid residues may be N-terminal or C-terminal to the serine/threonine kinase domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the serine/threonine kinase domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified
- 15 number.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, or all sixteen of the immunogenic epitopes shown in SEQ ID NO: 17 as residues: Ala-38 to Leu-43, Ser-48 to Glu-57, Asn-128 to Ser-139, Ser-169 to Asp-181, Pro-194 to Thr-199, Gln-220 to Gly-226, Pro-229 to Ala-234, Arg-243 to Leu-249, Pro-272 to Gln-277, Leu-280 to Asp-288, Asp-296 to Lys-311, Val-328 to Glu-334, Lys-356 to Ser-374, Asp-388 to Gln-396, Glu-467 to Leu-474, and Glu-487 to Gly-507. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed primarily in neutrophils and ovarian cancer tissue.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of the immune system, and ovarian cancer. Similarly, 5 polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, reproductive, cancerous and wounded 10 tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

15 The tissue distribution in neutrophils and ovarian cancer tissues, and the homology to kinase proteins, indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the immune system, and ovarian cancers. Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular 20 proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

25 Expression of this gene product in neutrophils suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is

expressed in cells of hematopoietic origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Polynucleotides, translation products and antibodies corresponding to this gene may be also used as an agent for immunological disorders including arthritis, 5 asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Expression of this gene product in neutrophils also strongly suggests a role for this protein in immune 10 function and immune surveillance.

Alternatively, the tissue distribution in ovarian cancer tissue indicates that this gene would be a good target for agonists or antagonists, particularly antibodies, which block binding of the receptor by its cognate ligand(s). Accordingly, preferred are antibodies and/or small molecules which specifically bind a portion of the translation product of this gene. Also 15 provided is a kit for detecting ovarian cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting ovarian cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found 20 in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation products, may show utility as a tumor marker and/or immunotherapy targets for the above 25 listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 5

Translation products corresponding to this gene share sequence homology with the murine NEK6 protein (See International Publication No. WO9966051), which is a protein thought to have serine/threonine kinase activity. Based upon the homology, it is anticipated
5 that these proteins will share at least some biological activity.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or all thirteen of the immunogenic epitopes shown in SEQ ID NO: 18 as residues: Leu-2 to Asp-11, Asn-31 to Ser-36, Ala-73 to Ser-82, Glu-107 to Ala-116, Gly-130 to Gln-138, Leu-145 to Trp-150,
10 Gln-158 to Gln-189, Ala-210 to Leu-217, Val-226 to Pro-237, Gln-257 to Phe-274, Ser-295 to Asn-300, Thr-313 to Gly-326, and Pro-329 to Glu-340. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind
15 these polypeptides.

This gene is expressed primarily in infant brain, cerebellum, colon, and colon cancer tissues.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a
20 biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of the neural and gastrointestinal systems, and cancer(s). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neural and
25 gastrointestinal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., brain, neural, colon, gastrointestinal,

cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

- 5 The tissue distribution in neural tissue, colon cancer tissue, and gastrointestinal tissues, and the homology to the murine NEK6 protein, indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the neural and gastrointestinal systems, as well as for the diagnosis, detection and/or treatment of colon cancer.
- 10 Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

The tissue distribution in colon tissue suggests that the protein product of this clone is useful for the diagnosis and/or treatment of disorders involving the gastrointestinal system.

- 15 This may include diseases associated with digestion and food absorption, as well as hematopoietic disorders involving the Peyer's patches of the small intestine, or other hematopoietic cells and tissues within the body. Further, there exists a potential role for this gene as a diagnostic marker or causative agent in the development of colon cancer, and cancer in general. Therefore, translation products corresponding to this gene would be a good
- 20 target for agonists or antagonists, particularly antibodies, which block binding of the receptor by its cognate ligand(s). Accordingly, preferred are antibodies and/or small molecules which specifically bind a portion of the translation product of this gene. Also provided is a kit for detecting testicular cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of
- 25 detecting colon cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual,

preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein.

- 5 Alternatively, the tissue distribution in neural tissues suggests that polynucleotides, translation products and antibodies corresponding to this gene are useful for the detection and/or treatment of neurodegenerative disease states and behavioural disorders such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder,
- 10 learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation products,
- 15 may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 6

- The translation product of this gene shares sequence homology with a number of kinase proteins (See Genbank Accession CAB76471, and International Publication No. WO9418328).

- The gene encoding the translation product of the present invention is a member of the TGF-beta receptor family of proteins. Proteins belonging to this family contain a cytoplasmic serine/threonine kinase domain. In order to identify these kinase domains, the following
- 25 consensus pattern has been developed:

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3). Preferred polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: IIHRDVKP DNILL (SEQ ID NO: 29). Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides. Further preferred are polypeptides comprising the serine/threonine kinase domain of SEQ ID NO: 29, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of SEQ ID NO: 19. The additional contiguous amino acid residues may be N-terminal or C-terminal to the serine/threonine kinase domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the serine/threonine kinase domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or all twelve of the immunogenic epitopes shown in SEQ ID NO: 19 as residues: Arg-91 to Asp-97, Asn-137 to Ser-143, Asn-174 to Cys-180, Arg-194 to Leu-223, Arg-239 to Pro-252, Trp-265 to Asn-270, Gly-304 to Gly-315, Thr-320 to Gly-331, Thr-344 to Cys-349, Gly-360 to Trp-372, Pro-374 to Gly-380, and Arg-400 to Ala-410. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed primarily in neural tissues, such as adult and infant brain tissues, and hypothalamus tissues, as well as in monocytes.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of the nervous and immune systems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a 5 number of disorders of the above tissues or cells, particularly of the nervous and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard 10 gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neural tissues indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the neural and immune systems.

15 Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

The tissue distribution suggests that the protein product of this clone is useful for the detection/treatment of neurodegenerative disease states and behavioural disorders such as 20 Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with 25 the developing embryo, or sexually-linked disorders.

Alternatively, the tissue distribution in monocytes suggests that this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of hematopoietic origin, this gene may play a 5 role in the survival, proliferation, and/or differentiation of hematopoietic cells in general, and may be of use in augmentation of the numbers of stem cells and committed progenitors. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation products, may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

10

FEATURES OF PROTEIN ENCODED BY GENE NO: 7

The translation product of this gene shares sequence homology with the human warts protein (See International Publication No. WO9915558), which is homologous to the Drosophila warts gene expression product and is thought to be involved in the regulation of 15 the proliferation and/or differentiation of epithelial cells, and suppression of tumour formation. Furthermore, the translation product of this gene shares sequence homology with the murine m-lats2 protein (See International Publication No. WO9630402), which is a putative protein-serine/threonine-kinase (PSTK) thought to inhibit cell proliferation, and to play a crucial role throughout development.

20 The gene encoding the translation product of the present invention is a member of the TGF-beta receptor family of proteins. Proteins belonging to this family contain a cytoplasmic serine/threonine kinase domain. In order to identify these kinase domains, the following consensus pattern has been developed:

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3). Preferred polypeptides of 25 the invention comprise, or alternatively consist of, the following amino acid sequence: FIHRDIKPNDNILI (SEQ ID NO: 30). Fragments and/or variants of these polypeptides, such

as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides. Further preferred are polypeptides comprising the serine/threonine kinase domain of SEQ ID NO: 30, 5 and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of SEQ ID NO: 20. The additional contiguous amino acid residues may be N-terminal or C-terminal to the serine/threonine kinase domain. Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the serine/threonine kinase domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified 10 number.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, or all seven of the immunogenic epitopes shown in SEQ ID NO: 20 as residues: His-76 to Gln-122, Ala-211 to Gly-219, Ser-236 to Ala-243, Asp-259 to Ser-274, Leu-281 to Glu-290, Arg-300 to Gly-316, and Ser-320 to Ser-329. 15 Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed in nine-week old embryonic tissues, and to a lesser extent in 20 smooth muscle tissues.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of musculo-skeletal tissues, and developmental disorders 25 and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell

type(s). For a number of disorders of the above tissues or cells, particularly of the developing systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developing, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another 5 tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in smooth muscle and embryonic tissues, and the homology to the warts and m-lats proteins, indicates that polynucleotides, translation products and 10 antibodies corresponding to this gene are useful for the diagnosis, detection and/or treatment of muscular and embryonic disorders, and cancers. The tissue distribution suggests that the protein product of this clone is useful for the diagnosis and treatment of cancer and other proliferative disorders.

Expression within embryonic tissue and other cellular sources marked by proliferating 15 cells suggests that this protein may play a role in the regulation of cellular division, or may play a role in the inhibition of the proliferation of particular cell types, such as cells of muscular tissues, for example. The tissue distribution in smooth muscle tissue indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions and pathologies of the cardiovascular system, such as heart disease, restenosis, atherosclerosis, 20 stroke, angina, thrombosis, and wound healing. Additionally, polynucleotides, translation products and antibodies corresponding to this gene may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and 25 differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may

also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation products, may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 8

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, or all twenty-five of the immunogenic epitopes shown in SEQ ID NO: 21 as residues: Lys-15 to Leu-21, Asp-27 to Met-40, Arg-43 to Asn-52, Pro-55 to Asp-89, Leu-105 to Asp-124, Glu-138 to Asp-150, Thr-157 to Asn-166, Arg-198 to Gly-208, Lys-216 to Glu-225, Ser-250 to Glu-258, Thr-266 to Gly-291, Lys-294 to Glu-299, Ile-329 to Cys-339, Asn-367 to Phe-373, Ser-410 to Val-416, Ile-420 to Leu-432, Thr-474 to Leu-485, Thr-488 to Arg-493, Ser-506 to Lys-515, Ser-521 to Met-526, Ile-530 to Ala-535, Glu-553 to Leu-566, Leu-581 to Leu-586, Asp-647 to Lys-652, and Glu-657 to Gly-665. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as 20 are antibodies that bind these polypeptides.

This gene is expressed primarily in infant brain, and fetal and embryonic tissues.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of neural and developing systems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neural and developing systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developing, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in infant brain tissue, and fetal and embryonic tissues, indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the neural and developing systems. Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

The tissue distribution in infant brain tissue suggests that polynucleotides, translation products and antibodies corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioural disorders such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

Alternatively, the tissue distribution in embryonic tissue suggests that this protein may play a role in the regulation of cellular division. Embryonic development involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this

protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 9

Translation products corresponding to this gene share sequence homology with CDC2-related protein kinase 7 (See Genbank Accession AAF36401). Based upon the homology, it is thought that these proteins will share at least some biological activities.

Preferred polypeptides of the present invention comprise, or alternatively consist of, 10 one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, or all fifteen of the immunogenic epitopes shown in SEQ ID NO: 22 as residues: Met-1 to Glu-13, Pro-34 to Thr-39, Ser-73 to Ser-82, Pro-89 to Lys-122, Ser-171 to Gln-178, Ala-192 to Pro-204, Leu-256 to Glu-280, Pro-290 to Pro-307, Pro-337 to Gly-343, Met-355 to Thr-375, Asp-381 to Gly-388, Glu-414 to Gly-422, Thr-464 to Glu-471, Gly-490 to Tyr-497, 15 and Lys-499 to Val-517. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed in testes tissue, neutrophils, T cells, and smooth muscle tissue. 20 Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of the reproductive, immune and cardiovascular systems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing 25 immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and

cardiovascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, immune, cardiovascular, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in testes, smooth muscle and immune tissues indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the reproductive, immune and cardiovascular systems. Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers. Similarly, agonistic antibodies directed against translation products corresponding to this gene may enable the inhibition of cellular proliferation.

The tissue distribution indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g., endocrine function, sperm maturation), as well as cancer. Therefore, polynucleotides, translation products and antibodies corresponding to this gene are useful in the treatment of male infertility and/or impotence. Polynucleotides, translation products and antibodies corresponding to this gene are also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, polynucleotides, translation products and antibodies corresponding to this gene are believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional

roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

The tissue distribution in smooth muscle tissue indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions and pathologies of the cardiovascular system, such as heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis, and wound healing. Expression of this gene product in T cells and neutrophils also strongly suggests a role for this protein in immune function and immune surveillance. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation products, may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 10

The gene encoding the translation product of the present invention is a member of the TGF-beta receptor family of proteins. Proteins belonging to this family contain a cytoplasmic serine/threonine kinase domain. In order to identify these kinase domains, the following consensus pattern has been developed:

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3). Preferred polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: LVHRDIKLKNVLL (SEQ ID NO: 31). Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides. Further preferred are polypeptides comprising the serine/threonine kinase domain of SEQ ID NO: 31, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of SEQ ID NO: 23. The additional contiguous amino acid residues may be N-terminal or C-terminal to the serine/threonine kinase domain. Alternatively, the additional contiguous amino acid

residues may be both N-terminal and C-terminal to the serine/threonine kinase domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number.

Preferred polypeptides of the present invention comprise, or alternatively consist of, 5 one, two, three, four, or all four of the immunogenic epitopes shown in SEQ ID NO: 23 as residues: Gly-20 to Ser-25, Val-62 to Arg-70, Trp-86 to Lys-92, and Leu-110 to Thr-126. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding 10 these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed primarily in melanocytes and microvascular endothelial cells.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not 15 limited to: diseases and/or disorders of skin and musculo-skeletal tissues. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the integumentary and musculo-skeletal systems, expression of this gene at significantly higher or lower levels may 20 be routinely detected in certain tissues or cell types (e.g., skin, musculo-skeletal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

25 The tissue distribution in melanocytes and microvascular endothelial cells indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful

for the diagnosis, detection and/or treatment of diseases and/or disorders of skin and musculo-skeletal systems. Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

5 The tissue distribution in melanocytes suggests that the protein product of this clone is useful for the treatment, diagnosis, and/or prevention of various skin disorders including congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e. keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and 10 Kaposi's sarcoma), injuries and inflammation of the skin (i.e. wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, uticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. Moreover, such disorders may predispose increased susceptibility to viral and 15 bacterial infections of the skin (i.e. cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, athlete's foot, and ringworm).

Alternatively, the tissue distribution in microvascular endothelial cells suggests that the protein product of this clone is useful for the diagnosis and/or treatment of disorders involving the vasculature. Elevated expression of this gene product by endothelial cells 20 suggests that it may play vital roles in the regulation of endothelial cell function; secretion; proliferation; or angiogenesis. Alternately, this may represent a gene product expressed by the endothelium and transported to distant sites of action on a variety of target organs. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation products, may show utility as a tumor marker and/or immunotherapy 25 targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 11

The translation product of this gene shares sequence homology with Human transforming growth factor-beta activated kinase TAK-1 (See Japanese Patent No. J09163990), which is thought to be important in activating the MAPK kinase by phosphorylation. The translation product of this gene also shares sequence homology with a number of protein kinases (See Genbank Accession AAA32779).

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or all thirteen of the immunogenic epitopes shown in SEQ ID NO: 24 as residues: Gln-27 to Gly-32, Asp-5 to Pro-54, Glu-77 to Ile-85, Asp-100 to Leu-105, Gln-135 to Arg-145, Arg-153 to Ile-158, Lys-170 to Leu-180, Glu-191 to Gly-203, Thr-246 to Thr-253, Leu-294 to Asp-300, Thr-363 to Ser-373, Gly-414 to Ile-420, and Lys-427 to Leu-441. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed primarily in smooth muscle and microvascular endothelial cells, as well as in colon cancer tissue and T cell lymphoma.

Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of the gastrointestinal and cardiovascular systems, and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the gastrointestinal and cardiovascular systems, and cancerous tissues, expression of this gene at significantly higher

or lower levels may be routinely detected in certain tissues or cell types (e.g., colon, musculo-skeletal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in
5 healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in smooth muscle, microvascular endothelial cells, T cell lymphoma and colon cancer tissue, in conjunction with the homology to a kinase activated by TGF-beta, as well as other kinase proteins, indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the diagnosis, detection
10 and/or treatment of diseases and/or disorders of the gastrointestinal and cardiovascular systems, and cancerous tissues such as colon cancer and T cell lymphoma. Polynucleotides and translation products corresponding to this gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

15 The tissue distribution in microvascular endothelial cells suggests that the protein product of this clone is useful for the diagnosis and/or treatment of disorders involving the vasculature. Expression of this gene product by endothelial cells suggests that it may play vital roles in the regulation of endothelial cell function; secretion; proliferation; or angiogenesis. The tissue distribution in smooth muscle tissue indicates that the protein
20 product of this gene is useful for the diagnosis and/or treatment of conditions and pathologies of the cardiovascular system, such as heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis, and wound healing.

Furthermore, the tissue distribution in colon cancer tissue and T cell lymphoma tissue suggests that polynucleotides, translation products and antibodies corresponding to this gene
25 are useful for the detection and/or treatment of colon cancer and T cell lymphomas, as well as cancers of other tissues where expression has been observed. The tissue distribution in colon

cancer tissue also indicates that this gene would be a good target for agonists or antagonists, particularly antibodies, which block binding of the receptor by its cognate ligand(s). Accordingly, preferred are antibodies and/or small molecules which specifically bind a portion of the translation product of this gene. Also provided is a kit for detecting colon 5 cancer and/or T cell lymphoma. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting colon cancer or T cell lymphoma in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found 10 in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein. Additionally, translation products corresponding to this gene, as well as antibodies directed against these translation products, may show utility as a tumor marker and/or immunotherapy targets for the above 15 listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 12

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, or both of the immunogenic epitopes shown in SEQ ID NO: 25 as residues: Ile-6 to 20 Ser-28 and Phe-69 to Phe-77. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

This gene is expressed in liver hepatoma tissue.
25 Therefore, polynucleotides and polypeptides of the invention, including antibodies, are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of diseases and conditions which include but are not limited to: liver hepatomas, and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or 5 cells, particularly of the liver, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., liver, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from 10 an individual not having the disorder.

The tissue distribution in liver hepatoma tissue indicates that polynucleotides, translation products and antibodies corresponding to this gene are useful for the diagnosis, detection and/or treatment of liver hepatomas, as well as cancers of other tissues where expression has been observed. Polynucleotides and translation products corresponding to this 15 gene may be involved in the inhibition of cellular proliferation, and thus may be useful in the prevention and/or elimination of cellular proliferative disorders, such as cancers.

The tissue distribution in liver hepatoma tissue indicates that this gene would be a good target for agonists or antagonists, particularly antibodies, which block binding of the receptor by its cognate ligand(s). Accordingly, preferred are antibodies and/or small 20 molecules which specifically bind a portion of the translation product of this gene. Also provided is a kit for detecting liver hepatomas. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting liver hepatomas in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from 25 the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is

serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein.

TABLE 1

5

| Gene No. | cDNA Clone ID | ATCC Deposit No:Z and Date | Vector | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT Start Codon | AA SEQ ID NO: Y | Last AA of ORF |
|----------|---------------|----------------------------|---------------|-----------------|---------------|---------------------|---------------------|-------------------|-----------------|----------------|
| 1 | HNTSY52 | PTA539 08/13/99 | pSport1 | 2 | 4087 | 1 | 4087 | 266 | 14 | 302 |
| 2 | HDPSM48 | PTA539 08/13/99 | pCMVSport 3.0 | 3 | 2622 | 1 | 2622 | 170 | 15 | 800 |
| 3 | HMTBH67 | PTA539 08/13/99 | pCMVSport 3.0 | 4 | 3283 | 1 | 3283 | 218 | 16 | 416 |
| 4 | HDPGX45 | PTA539 08/13/99 | pCMVSport 3.0 | 5 | 3200 | 1 | 3200 | 179 | 17 | 534 |
| 5 | HE9OS42 | PTA539 08/13/99 | Uni-ZAP XR | 6 | 2333 | 1 | 2333 | 714 | 18 | 365 |
| 6 | HTEMH65 | PTA539 08/13/99 | Uni-ZAP XR | 7 | 1490 | 1 | 1490 | 90 | 19 | 429 |
| 7 | HE9PQ20 | PTA539 08/13/99 | Uni-ZAP XR | 8 | 1501 | 1 | 1501 | 16 | 20 | 344 |
| 8 | HFIHY04 | PTA539 08/13/99 | pSport1 | 9 | 2331 | 852 | 2331 | 88 | 21 | 665 |
| 9 | HWAAP61 | PTA539 08/13/99 | pCMVSport 3.0 | 10 | 3515 | 1 | 3515 | 204 | 22 | 519 |
| 10 | HMELC05 | PTA539 08/13/99 | Lambda ZAP II | 11 | 1258 | 1 | 1258 | 189 | 23 | 126 |
| 11 | HTXPX58 | PTA539 08/13/99 | Uni-ZAP XR | 12 | 3240 | 1524 | 3240 | 879 | 24 | 471 |
| 12 | HLDAE46 | PTA539 08/13/99 | pCMVSport 3.0 | 13 | 793 | 1 | 793 | 272 | 25 | 78 |

Table I summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from 10 partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The

overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple 5 different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No:" The deposited plasmid contains all of these sequences, reflected by the 10 nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative methionine start codon (if present) is identified as "5' NT of Start Codon." Similarly , the nucleotide position of SEQ ID NO:X of the predicted signal sequence (if present) is identified as "5' NT of First AA of Signal Pep."

15 The translated amino acid sequence, beginning with the first translated codon of the polynucleotide sequence, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

20 SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X has uses including, but not limited to, in designing nucleic acid hybridization probes 25 that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in a deposited plasmid. These probes will also hybridize to nucleic acid molecules in biological

samples, thereby enabling a variety of forensic and diagnostic methods of the invention.

Similarly, polypeptides identified from SEQ ID NO:Y have uses that include, but are not limited to generating antibodies, which bind specifically to the secreted proteins encoded by the cDNA clones identified in Table 1.

5 Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, 10 even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide 15 sequence identified as SEQ ID NO:X, and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited plasmid can readily be determined by sequencing the deposited plasmid in accordance with known methods.

20 The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular plasmid can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

Also provided in Table 1 is the name of the vector which contains the cDNA plasmid. Each vector is routinely used in the art. The following additional information is provided for convenience.

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128,256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res.* 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res.* 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies* 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene.

Vectors pSport1, pCMV Sport 1.0, pCMV Sport 2.0 and pCMV Sport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus* 15:59 (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res.* 16:9677-9686 (1988) and Mead, D. et al., *Bio/Technology* 9: (1991).

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or a deposited plasmid (cDNA plasmid:Z). The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein.

Such methods include, but are not limited to, preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or cDNA plasmid:Z, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X and/or cDNA plasmid:Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the cDNA in cDNA plasmid:Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X and/or a polypeptide encoded by the cDNA in cDNA plasmid:Z, are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the complement of the coding strand of the cDNA in cDNA plasmid:Z.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would

unduly burden the disclosure of this application. Accordingly, preferably excluded from SEQ ID NO:X are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 and the final nucleotide minus 15 of SEQ ID NO:X, b is an integer of 15 to the final nucleotide of SEQ ID NO:X, where both a 5 and b correspond to the positions of nucleotide residues shown in SEQ ID NO:X, and where b is greater than or equal to a + 14.

RACE Protocol For Recovery of Full-Length Genes

Partial cDNA clones can be made full-length by utilizing the rapid amplification of 10 cDNA ends (RACE) procedure described in Frohman, M.A., et al., Proc. Nat'l. Acad. Sci. USA, 85:8998-9002 (1988). A cDNA clone missing either the 5' or 3' end can be reconstructed to include the absent base pairs extending to the translational start or stop codon, respectively. In some cases, cDNAs are missing the start of translation, therefor. The following briefly describes a modification of this original 5' RACE procedure. Poly A+ or 15 total RNA is reverse transcribed with Superscript II (Gibco/BRL) and an antisense or complementary primer specific to the cDNA sequence. The primer is removed from the reaction with a Microcon Concentrator (Amicon). The first-strand cDNA is then tailed with dATP and terminal deoxynucleotide transferase (Gibco/BRL). Thus, an anchor sequence is produced which is needed for PCR amplification. The second strand is synthesized from the 20 dA-tail in PCR buffer, Taq DNA polymerase (Perkin-Elmer Cetus), an oligo-dT primer containing three adjacent restriction sites (XhoI, SalI and ClaI) at the 5' end and a primer containing just these restriction sites. This double-stranded cDNA is PCR amplified for 40 cycles with the same primers as well as a nested cDNA-specific antisense primer. The PCR products are size-separated on an ethidium bromide-agarose gel and the region of gel 25 containing cDNA products the predicted size of missing protein-coding DNA is removed. cDNA is purified from the agarose with the Magic PCR Prep kit (Promega), restriction

digested with Xhol or Sall, and ligated to a plasmid such as pBluescript SKII (Stratagene) at XhoI and EcoRV sites. This DNA is transformed into bacteria and the plasmid clones sequenced to identify the correct protein-coding inserts. Correct 5' ends are confirmed by comparing this sequence with the putatively identified homologue and overlap with the 5 partial cDNA clone. Similar methods known in the art and/or commercial kits are used to amplify and recover 3' ends.

Several quality-controlled kits are commercially available for purchase. Similar reagents and methods to those above are supplied in kit form from Gibco/BRL for both 5' and 10 3' RACE for recovery of full length genes. A second kit is available from Clontech which is a modification of a related technique, SLIC (single-stranded ligation to single-stranded cDNA), developed by Dumas et al., Nucleic Acids Res., 19:5227-32 (1991). The major differences in procedure are that the RNA is alkaline hydrolyzed after reverse transcription and RNA ligase is used to join a restriction site-containing anchor primer to the first-strand cDNA. This obviates the necessity for the dA-tailing reaction which results in a polyT 15 stretch that is difficult to sequence past.

An alternative to generating 5' or 3' cDNA from RNA is to use cDNA library double-stranded DNA. An asymmetric PCR-amplified antisense cDNA strand is synthesized with an 20 antisense cDNA-specific primer and a plasmid-anchored primer. These primers are removed and a symmetric PCR reaction is performed with a nested cDNA-specific antisense primer and the plasmid-anchored primer.

RNA Ligase Protocol For Generating The 5' or 3' End Sequences To Obtain Full Length Genes

Once a gene of interest is identified, several methods are available for the 25 identification of the 5' or 3' portions of the gene which may not be present in the original cDNA plasmid. These methods include, but are not limited to, filter probing, clone

enrichment using specific probes and protocols similar and identical to 5' and 3'RACE. While the full length gene may be present in the library and can be identified by probing, a useful method for generating the 5' or 3' end is to use the existing sequence information from the original cDNA to generate the missing information. A method similar to 5'RACE is 5 available for generating the missing 5' end of a desired full-length gene. (This method was published by Fromont-Racine et al., Nucleic Acids Res., 21(7):1683-1684 (1993)). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcript and a primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of 10 interest, is used to PCR amplify the 5' portion of the desired full length gene which may then be sequenced and used to generate the full length gene. This method starts with total RNA isolated from the desired source, poly A RNA may be used but is not a prerequisite for this procedure. The RNA preparation may then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the 15 later RNA ligase step. The phosphatase if used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation can then be used as a template for first strand cDNA 20 synthesis using a gene specific oligonucleotide. The first strand synthesis reaction can then be used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the TGF-beta receptor gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the relevant TGF-beta receptor gene.

The present invention is also directed to polynucleotide fragments of the polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers to a polynucleotide having a nucleic acid sequence which: is a portion of the cDNA contained in cDNA plasmid:Z or encoding the polypeptide encoded by the cDNA
5 contained in cDNA plasmid:Z; is a portion of the polynucleotide sequence in SEQ ID NO:X or the complementary strand thereto; is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; or is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably
10 at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, at least about 100 nt, at least about 125 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from, for example, the sequence contained in the cDNA in cDNA plasmid:Z, or the nucleotide sequence shown in SEQ ID NO:X or the complementary stand
15 thereto. In this context "about" includes the particularly recited value, or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 150, 175, 200, 250, 500, 600, 1000, or 2000 nucleotides in length) are also encompassed by the invention.
20 Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300,
25 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050,

2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-
2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800,
2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-
3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550,
5 3551-3600, 3601-3650, 3651-3700, 3701-3750, 3751-3800, 3801-3850, 3851-3900, 3901-
3950, 3951-4000, 4001-4050, and/or 4051-4087 of SEQ ID NO:X, or the complementary
strand thereto. In this context “about” includes the particularly recited range or a range larger
or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.
Preferably, these fragments encode a polypeptide which has a functional activity (e.g.
10 biological activity) of the polypeptide encoded by a polynucleotide of which the sequence is a
portion. More preferably, these fragments can be used as probes or primers as discussed
herein. Polynucleotides which hybridize to one or more of these fragments under stringent
hybridization conditions or alternatively, under lower stringency conditions, are also
encompassed by the invention, as are polypeptides encoded by these polynucleotides or
15 fragments.

Moreover, representative examples of polynucleotide fragments of the invention,
include, for example, fragments comprising, or alternatively consisting of, a sequence from
about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-
400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900,
20 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300,
1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-
1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050,
2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-
2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800,
25 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-
3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550,

3551-3600, 3601-3650, 3651-3700, 3701-3750, 3751-3800, 3801-3850, 3851-3900, 3901-3950, 3951-4000, 4001-4050, and/or 4051-4087 of the cDNA nucleotide sequence contained in cDNA plasmid:Z, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides,

5 at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g. biological activity) of the polypeptide encoded by the cDNA nucleotide sequence contained in cDNA plasmid:Z. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these fragments under stringent hybridization conditions, or alternatively, under

10 lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, and/or encoded by the cDNA in cDNA plasmid:Z. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, an amino acid sequence from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, and/or 781-800 of the coding region of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or

values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results
5 in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are
10 removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid
15 residues may often evoke an immune response.

Accordingly, polypeptide fragments of the invention include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can
20 be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

25 The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed

herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in cDNA plasmid:Z). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues
5 in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of
10 the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular
15 polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

20 Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in cDNA plasmid:Z). In particular, C-terminal deletions may be described by the general
25 formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Polynucleotides

encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides 5 having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID NO:Y), and/or the cDNA in cDNA plasmid:Z, and/or the complement thereof, where n and m are integers as described above. Polynucleotides encoding these polypeptides, including 10 fragments and/or variants, are also encompassed by the invention.

Any polypeptide sequence contained in the polypeptide of SEQ ID NO:Y, encoded by the polynucleotide sequences set forth as SEQ ID NO:X, or encoded by the cDNA in cDNA plasmid:Z may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of 15 SEQ ID NO:X or the cDNA in cDNA plasmid:Z may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; <http://www.dnastar.com/>).

Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, 20 turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides 25 of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

- Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be 5 used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.
- 10 Preferred polypeptide fragments of the invention are fragments comprising, or alternatively, consisting of, an amino acid sequence that displays a functional activity (e.g. biological activity) of the polypeptide sequence of which the amino acid sequence is a fragment. By a polypeptide displaying a "functional activity" is meant a polypeptide capable of one or more known functional activities associated with a full-length protein, such as, for 15 example, biological activity, antigenicity, immunogenicity, and/or multimerization, as described supra.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments 20 may include an improved desired activity, or a decreased undesirable activity.

In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

25 The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide sequence shown in SEQ ID NO:Y, or an epitope

of the polypeptide sequence encoded by the cDNA in cDNA plasmid:Z, or encoded by a polynucleotide that hybridizes to the complement of an epitope encoding sequence of SEQ ID NO:X, or an epitope encoding sequence contained in cDNA plasmid:Z under stringent hybridization conditions, or alternatively, under lower stringency hybridization, as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to this complementary strand under stringent hybridization conditions, or alternatively, under lower stringency hybridization conditions, as defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred 5 polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. 10 Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies 15 according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic 20 epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear 25 epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention and immunogenic and/or antigenic epitope fragments thereof can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first

two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

10 Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the

15 fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K.

20 Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for

convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the
5 polypeptides of the present invention.

Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed
10 in human cell lines (Janknecht et al., Proc. Natl. Acad. Sci. USA 88:8972- 897 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto
15 Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities
20 of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco,
25 Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides

corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the 5 encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous 10 molecules.

Polynucleotide and Polypeptide Variants

The invention also encompasses TGF-beta receptor variants. The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the 15 complementary strand thereto, and/or the cDNA sequence contained in cDNA plasmid:Z.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X and/or a polypeptide sequence encoded by the cDNA in cDNA plasmid:Z.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide 20 or polypeptide of the present invention, but retaining properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence 25 selected from the group consisting of : (a) a nucleotide sequence encoding a TGF-beta receptor polypeptide having an amino acid sequence as shown in the sequence listing and

described in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (b) a nucleotide sequence encoding a mature TGF-beta receptor polypeptide having the amino acid sequence as shown in the sequence listing and described in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (c) a nucleotide sequence encoding a biologically active fragment of a TGF-beta receptor
5 polypeptide having an amino acid sequence shown in the sequence listing and described in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (d) a nucleotide sequence encoding an antigenic fragment of a TGF-beta receptor polypeptide having an amino acid sequence shown in the sequence listing and described in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (e)
10 a nucleotide sequence encoding a TGF-beta receptor polypeptide comprising the complete amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (f) a nucleotide sequence encoding a mature TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (g) a nucleotide sequence encoding a biologically active fragment of a TGF-beta receptor polypeptide having an amino acid
15 sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (h) a nucleotide sequence encoding an antigenic fragment of a TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA in cDNA plasmid:Z; (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h),
20 above.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), or (i) above. Polypeptides encoded by these nucleic acid
25 molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise, or alternatively, consist of a

polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), (h), or (i), above. Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency 5 conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

Another aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of : (a) a nucleotide sequence encoding a TGF-beta receptor polypeptide having an amino acid sequence as shown in the sequence listing and described in Table 1; (b) a nucleotide sequence encoding a mature TGF-beta receptor polypeptide having the amino acid sequence as shown in the sequence listing and described in Table 1; (c) a nucleotide sequence encoding a biologically active fragment of a TGF-beta receptor polypeptide having an amino acid sequence shown in the sequence listing and described in Table 1; (d) a nucleotide sequence encoding an antigenic fragment of a TGF-beta receptor polypeptide having an amino acid sequence shown in the sequence listing and described in Table 1; (e) a nucleotide sequence encoding a TGF-beta receptor polypeptide comprising the complete amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (f) a nucleotide sequence 10 encoding a mature TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (g) a nucleotide sequence encoding a biologically active fragment of a TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (h) a nucleotide sequence encoding 15 an antigenic fragment of a TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (i) a nucleotide sequence encoding a biologically active fragment of a TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (j) a nucleotide sequence encoding 20 an antigenic fragment of a TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (k) a nucleotide sequence encoding a biologically active fragment of a TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (l) a nucleotide sequence encoding 25 an antigenic fragment of a TGF-beta receptor polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1;

described in Table 1; (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 5 97%, 98%, 99% or 100%, identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), or (i) above. Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, 10 under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), (h), or (i), above. Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

15 The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the cDNA in cDNA plasmid:Z, and/or polypeptide fragments of any of 20 these polypeptides (e.g., those fragments described herein). Polynucleotides which hybridize to the complement of the nucleic acid molecules encoding these polypeptides under stringent hybridization conditions or alternatively, under lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" 25 to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide

sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another 5 nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence referred to in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is 10 at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the 15 algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identiy are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining 20 Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the lenght of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, 25 not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3'

ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment.

- 5 This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of
10 manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and
15 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which
20 are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95%
25 "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that

the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or 5 substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 10 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence referred to in Table 1 or a fragment thereof, the amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X or a fragment thereof, or to the amino acid sequence encoded by the cDNA in cDNA plasmid:Z, or a fragment thereof, can be determined conventionally using known computer programs. A preferred method for determining the best overall match 15 between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237- 245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 20 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal 25 deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of

the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues 5 to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues 10 of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the 15 subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the 20 FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. 25 Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the

query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce 5 silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which less than 50, less than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide 10 variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes 15 II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, 20 variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, as discussed herein, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after 25 deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma

exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol.

5 Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1 α . They used random mutagenesis to generate over 3,500 individual IL-1 α mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological 10 activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, as discussed herein, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more

15 biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be 20 determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show a functional activity (e.g. biological activity) of the polypeptide of the invention, of which they are a variant. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

25 The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein,

(e.g., encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion), irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid
5 molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al.,
10 Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having functional activity of a polypeptide of
15 the invention.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA in cDNA plasmid:Z, the nucleic acid
20 sequence referred to in Table 1 (SEQ ID NO:X), or fragments thereof, will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate
25 variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less

likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences:

- 5 Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

- 15 The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for
20 biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or

hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr,

5 Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the 10 stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

15 For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 20 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

25 A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course it is highly preferable for a polypeptide to have an amino acid

- sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, and/or the amino acid sequence encoded by the cDNA in cDNA plasmid:Z which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific 5 embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein), an amino acid sequence encoded by SEQ ID NO:X or fragments thereof, and/or the amino acid sequence encoded by cDNA plasmid:Z or fragments thereof, is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.
- 10 As discussed herein, any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on 15 trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker 20 sequences.

In certain preferred embodiments, proteins of the invention comprise fusion proteins wherein the polypeptides are N and/or C-terminal deletion mutants. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides 25 having the amino acid sequence of the specific N- and C-terminal deletions mutants. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also

encompassed by the invention.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to 5 improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

10 As one of skill in the art will appreciate, polypeptides of the present invention of the present invention and the epitope-bearing fragments thereof described above can be combined with heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with heterologous polypeptide sequences, for example, the polypeptides of the present invention may be fused with the constant domain of 15 immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the 20 heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

25

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells.

Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, 5 pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, 10 pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, 15 electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell 20 cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

25 Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or

cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be 5 glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after 10 translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine 15 is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express polypeptides of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can 20 metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (AOX1) is highly active. In the presence of methanol, alcohol oxidase produced from 25 the AOX1 gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast* 5:167-77 (1989); Tschopp, J.F., et al., *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present

invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichia* yeast system essentially as described in "Pichia Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous

polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 5 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)).

10 For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-
15 aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib,
2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline,
hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-
butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer
amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids,
20 and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L
(levorotary).

The invention encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic
25 cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to

specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the

ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein.

- 5 There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which 10 an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is 15 attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the 20 reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus 25 modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available

for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, 5 trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least 10 tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or an amino acid sequence encoded by SEQ ID NO:X or the complement of SEQ ID NO:X, and/or an amino acid sequence encoded by 15 cDNA plasmid:Z (including fragments, variants, splice variants, and fusion proteins, corresponding to these as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer 20 containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a 25 homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric
5 multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example,
10 homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other
15 embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, or contained in a polypeptide encoded by SEQ ID NO:X, and/or the cDNA plasmid:Z). In one instance, the covalent associations are cross-linking between cysteine
20 residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the heterologous
25 sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous

sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant

protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

- 5 In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.
- 10 The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention
- 15 may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of
- 20 the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent
- 25 Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to

immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or 5 subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain 10 antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are 15 human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example 20 in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid 25 support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893;

4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or
5 specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size in contiguous amino acid residues. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that
10 specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at
15 least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than
20 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the
25 specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which

hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at

least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Lautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in

immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

5 As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused
10 or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment
15 does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known
20 techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various
25 procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to

induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic 5 polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a 10 combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term 15 "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma 20 technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any 25 suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then

assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal
5 antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

10 Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

15 For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial
20 antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the
25 phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J.

Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 5 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison,

Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having 5 one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the 10 interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for 15 example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

20 Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 25 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont,

CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human 5 monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using techniques 10 well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" 15 the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

20 *Polynucleotides Encoding Antibodies*

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an 25 antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols

in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain
5 variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework
10 regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of
15 the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or
20 more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a
25 mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra,

a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and

translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, 5 operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the 10 transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains 15 may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent 20 cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with 25 recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing

antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free

glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells.

- 5 The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized.

- 10 In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk,
15 Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can
20 be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

- 25 In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have

- characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the
- 5 primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.
- 10 For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.
- 15 This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.
- 20 A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can

be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid
5 (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993);
Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem.
10 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known
in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.),
Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters
15 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons,
NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene
20 amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3.
(Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol.
25 Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single 5 vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

10 Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or 15 by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or 20 portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. 25 For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of

the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 5 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated 10 to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can 15 form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; 20 Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Zheng et al., *J. Immunol.* 154:5590-5600 (1995); and Vil et al., *Proc. Natl. Acad. Sci. USA* 89:11337-11341(1992) (said references incorporated by reference in their entireties).

As discussed, *supra*, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody 25 portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be

fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988)).

5 The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, 10 improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to 15 identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995)).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 20 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the “HA” tag, which corresponds to an epitope derived from the influenza hemagglutinin protein 25 (Wilson et al., Cell 37:767 (1984)) and the “flag” tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be 5 facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or 10 fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic 15 group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 20 ^{125}I , ^{131}I , ^{111}In or ^{99}Tc .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytoidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, 25 gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone,

mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., 5 mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents 10 (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin 15 such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, 20 International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

25 Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not

limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types.

Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" 5 with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host 10 Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

15 The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin 20 reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are 25 described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium 5 vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One 10 of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

15 Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer 20 (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ³²P or ¹²⁵I) diluted in blocking buffer, 25 washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the

signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate
5 with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable
10 compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the
15 art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-
antigen interaction can be determined by competitive binding assays. One example of a
competitive binding assay is a radioimmunoassay comprising the incubation of labeled
20 antigen (e.g., ³H or ¹²⁵I) with the antibody of interest in the presence of increasing amounts
of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The
affinity of the antibody of interest for a particular antigen and the binding off-rates can be
determined from the data by scatchard plot analysis. Competition with a second antibody
25 can also be determined using radioimmunoassays. In this case, the antigen is incubated with
antibody of interest conjugated to a labeled compound (e.g., ³H or ¹²⁵I) in the presence of
increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth

factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, 5 immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

10 It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or 15 polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

20

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, 25 by way of gene therapy. Gene therapy refers to therapy performed by the administration to a

subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

5 For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can
10 be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host.
15 In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote
20 homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and
25 light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene 5 therapy.

In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become 10 intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the 15 nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another 20 embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 25 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdrl* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy.

Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells,

endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood,
5 peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for
10 therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

15 In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested
20 in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art
25 including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of

a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

5 *Therapeutic/Prophylactic Administration and Composition*

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, 20 J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In

addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary 5 administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, 10 topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

15 In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

20 In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise 25 (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger

and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of 5 the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid 10 encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or 15 transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions 20 comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle 25 with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic

origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include
5 starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release
10 formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W.
15 Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine
20 procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage
25 form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent.

Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

- 5 The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.
- 10 The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend
- 15 on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of

antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of 5 the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

10 *Diagnosis and Imaging*

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant 15 expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant 20 expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in 25 the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high

amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby

5 preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, et al., *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression

10 include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

15 One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of

20 interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a

25 particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the

amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the 5 case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 10 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled 15 molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial 20 diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but 25 are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument.

- 5 In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

10 *Kits*

- The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate,
- 15 a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).
- 20

- In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least

one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an

enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

15 Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 1 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 5 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the 10 physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian 15 Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50- 20 500 potential causative genes.

Thus, once coinheritance is established, differences in a polynucleotide of the 25 invention and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using the polynucleotides of the invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

5 Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a
10 disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention
15 and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a related disorder, including, for example, diagnosis of a tumor,
20 has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotides of the invention" is intended
25 qualitatively or quantitatively measuring or estimating the level of the polypeptide of the invention or the level of the mRNA encoding the polypeptide of the invention in a first

- biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or 5 mRNA level, the standard being taken from a second biological sample obtained from an individual not having the related disorder or being determined by averaging levels from a population of individuals not having a related disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.
- 10 By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains polypeptide of the present invention or the corresponding mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and tissue sources found to express the polypeptide of 15 the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a 20 solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the invention attached may be used to identify polymorphisms between the isolated polynucleotide sequences of the invention, with polynucleotides isolated 25 from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive

disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced *supra* are hereby incorporated by reference in their entirety herein.

5 The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA
10 analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P.
E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M.
Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S.
15 K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency
20 conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (*T_{sub.m}*) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA
25 means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic 5 leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in *Neoplastic Diseases of the Blood*, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification 15 of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., *supra*) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., *supra*) Indeed, the human counterparts of the oncogenes involved in some 20 animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., *supra*)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580). 25 However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding

mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not be limited to treatment of proliferative disorders of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide of the present invention can be used to control gene expression through triple helix formation or through antisense DNA or RNA.

10 Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary
15 DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense
20 Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of polypeptide of the present invention antigens. Both
25 techniques are effective in model systems, and the information disclosed herein can be used

to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort 5 to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological 10 samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, 15 making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying 20 and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

25 Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues,

- e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify
- 5 individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.
- 10 There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue
- 15 cultures for contamination.

The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g.,

20 immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention and/or cancerous and/or wounded tissues) or bodily fluids (e.g., serum,

25 plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder,

relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby an increase or decrease in the assayed gene expression level compared to the standard expression level is indicative of a disorder.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

15 Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for

detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium 5 (^{115m}In , ^{113m}In , ^{112}In , ^{111}In), and technetium (^{99}Tc , ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying levels of polypeptide of the present invention in a biological 10 sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be 15 incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , ^{99m}Tc , ^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{115m}In , ^{113m}In , ^{112}In , ^{111}In), and technetium (^{99}Tc , ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru), a radio-opaque substance, or a material 20 detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In 25 the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected

will normally range from about 5 to 20 millicuries of ^{99m}Tc . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which express the polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and 5 Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are 10 associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

15 In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of 20 toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria 25 toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera

toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi , or other radioisotopes such as, for example, ^{103}Pd , ^{133}Xe , ^{131}I , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{85}Sr , ^{32}P , ^{35}S , ^{90}Y , ^{153}Sm , ^{153}Gd , ^{169}Yb , ^{51}Cr , ^{54}Mn , ^{75}Se , ^{113}Sn , $^{90}\text{Yttrium}$, ^{117}Tin , $^{186}\text{Rhenium}$, $^{166}\text{Holmium}$, and $^{188}\text{Rhenium}$; 5 luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 10 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a polypeptide of the present invention in cells or body fluid 15 of an individual; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may 20 provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat or prevent 25 diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders,

cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for 5 hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the 10 immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described supra, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration 15 of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise 20 antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Belldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The

polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences 5 that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for 10 example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and 15 pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, 20 such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b- 25 actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

5 The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular 10 fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection 15 into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

20 For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate 25 and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be
5 delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

10 The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic
15 (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA
20 (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy]propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly
25 useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is

herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion

through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The 5 various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication 10 of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome 15 and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+} -EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta (1975) 394:483; Wilson et al., Cell (1979) 17:77); ether injection (Deamer, D. and Bangham, A., Biochim. Biophys. Acta 20 (1976) 443:629; Ostro et al., Biochem. Biophys. Res. Commun. (1977) 76:836; Fraley et al., Proc. Natl. Acad. Sci. USA (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., Proc. Natl. Acad. Sci. USA (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA (1978) 75:145; Schaefer-Ridder et al., Science (1982) 215:166), which are herein 25 incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. 10 WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding a polypeptide of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO₄ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express a polypeptide of the present invention.

5 In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome,
10 thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis. 109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991)
15 Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 20 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to
25

Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line 5 to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an 10 adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into 15 non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include 20 all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into 25 packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to

transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.

- Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989)).
- This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes,

viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

5 The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

Preferably, the polynucleotide encoding a polypeptide of the present invention
10 contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized
15 using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biostatic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene
20 in the rat livers (Kaneda et al., Science 243:375 (1989)).
25

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters 5 and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

10 Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, 15 oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 89:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of 20 withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a 25 number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its

severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

5 Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

Biological Activities

10 Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or
15 antagonists could be used to treat the associated disease.

Members of the TGF-beta receptor family of proteins are believed to be involved in biological activities associated with extracellular matrix production, inflammatory responses, hematopoietic and immune system responses, developmental regulation, and neoplastic disorders. Accordingly, compositions of the invention (including polynucleotides,
20 polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, detection and/or treatment of diseases and/or disorders associated with aberrant TGF-beta and TGF-beta receptor activities. In preferred embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, detection and/or treatment of
25 diseases and/or disorders relating to neoplastic disorders (e.g., cancers, and/or as described under "Hyperproliferative Disorders" and "Diseases at the Cellular Level" below), extracellular matrix production, immune system responses (e.g., inflammatory response, and/or as described under "Immune activity" below), and developmental regulation. Thus,

polynucleotides, translation products and antibodies of the invention are useful in the diagnosis, detection and/or treatment of diseases and/or disorders associated with activities that include, but are not limited to, extracellular matrix production, inflammatory responses, hematopoietic and immune system responses, developmental regulation, and neoplastic disorders.

More generally, polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, detection and/or treatment of diseases and/or disorders associated with the following systems.

10 Immune Activity

A polypeptide or polynucleotide, or agonists or antagonists of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. Polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g.

agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, 5 thrombocytopenia, or hemoglobinuria.

Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, or agonists or antagonists of the 10 present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in 15 the treatment of heart attacks (infarction), strokes, or scarring.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the 20 host tissue. Therefore, the administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected include, but are not 25 limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's

Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and 5 autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group 10 incompatibility.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this 15 case, the foreign transplanted immune cells destroy the host tissues. The administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides or polypeptides, or agonists or antagonists of the present 20 invention may also be used to modulate inflammation. For example, polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with 25 infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute

rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

5 Polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may 10 proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response.

15 Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by Polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive 20 system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention.

25 Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura,

sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit
5 aberrant cellular division, by gene therapy using the present invention, and/or protein fusions
or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

10 Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said
15 polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only
20 proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As
25 such the beneficial therapeutic affect of the present invention may be expressly modulated

(i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

The polynucleotides encoding a polypeptide of the present invention may be administered along with other polynucleotides encoding an angiogenic protein. Examples of 5 angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide 10 synthase.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the 15 prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in 20 vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 25 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated

by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus 5 will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell 10 proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, 15 affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. 20 Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell 25 growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal 5 antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated 10 by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are 15 useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth 20 factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing 25 antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragement thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or

polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

5 Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-
10 associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

15 Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-
20 mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the
25 expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for

example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and

ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

5 Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

10 Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

15 Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomas, bacillary angiomas, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, 20 Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein 25 occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia

telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart 5 aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include carotid artery diseases, cerebral amyloid 10 angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular 15 leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms.

Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, 20 carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, 25 hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biostatic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are 10 described in more detail herein.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 15 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated 20 angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 25 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743

(1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

5 The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid
10 tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist
15 of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum,
20 cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors,
25 and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists

may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary 5 according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular 10 angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; 15 myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for 20 treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to 25 prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of

hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal
5 neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrobulbar fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy,
10 retinoblastoma, retrobulbar fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalm.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

15 Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood
20 vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacifies. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma,
25 herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any

cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimetic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimetic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections

might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrothalental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreous injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis,

angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated with the the
5 polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal
10 graft rejection, neovascular glaucoma, retroental fibroplasia, rubeosis, retinoblastoma, and uvetitis, delayed wound healing, endometriosis, vascluogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization,
15 telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

20 In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal
25 implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

- Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a
- 5 compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale.
- 10 Within yet other aspects of the present invention, surgical meshes which have been coated with anti-angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide
- 15 support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within

20 one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-

25 angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of 5 a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives 10 thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form 15 transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, 20 sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetone and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide 25 complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV)

oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, 5 molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetone. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine 10 sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, 15 alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium 20 Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and 25 metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include cancers (such as follicular lymphomas, carcinomas 5 with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune 10 disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, 15 polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of 20 malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, 25 Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma,

chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma,
5 adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma,
10 astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendrogioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, 15 include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and
20 rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avascular grafts, Blair-Brown grafts, bone graft, brepheplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as

agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention,

could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine,
5 respectively. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the production of mucus throughout the
10 gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associate with the under expression.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage.
15 For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat
20 or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.
25

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., 5 acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, 10 could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

15 Neurological Diseases

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or 20 antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include 25 brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase

complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis, cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache, migraine, dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, 25 encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes

MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, Hallervorden-Spatz Syndrome, hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as 5 hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, 10 Necrotizing Hemorrhagic Encephalomyelitis, Visna, cerebral malaria, meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis. Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis, Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome, Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as 15 Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uvelemingoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie) cerebral toxoplasmosis, 20 central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral sceloris which includes adrenoleukodystrophy, 25 encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic

encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy

5 such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan

10 Syndrome, Maple Syrup Urine Disease, mucolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes

15 hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta, hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraparesis, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial

20 Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired

25 Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative

disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia, Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve

paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as

5 Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, Diabetic neuropathies such as diabetic foot, nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial

10 neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial

15 Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

Infectious Disease

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting

20 an immune response.

25

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g.,

5 Corynebacterium, Mycobacterium, Nocardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella

10 typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis,

15 Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's

20 Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g.,

25 cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat

or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, Diphteria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated or
5 detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present
invention include, but not limited to, the following families or class: Amebiasis, Babesiosis,
Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis,
Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and
Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium
10 malariae and Plasmodium ovale). These parasites can cause a variety of diseases or
symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal
disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g.,
AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or
polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of
15 these symptoms or diseases.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present
invention of the present invention could either be by administering an effective amount of a
polypeptide to the patient, or by removing cells from the patient, supplying the cells with a
polynucleotide of the present invention, and returning the engineered cells to the patient (ex
20 vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be
used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

Polynucleotides or polypeptides, as well as agonists or antagonists of the present
25 invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration
of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to

repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

5 Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

10 Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could
15 be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associatedwith vascular insufficiency, surgical, and traumatic wounds.

20 Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system
25 diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic

lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

Chemotaxis

5 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or
10 abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body.

15 For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could
20 also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind
25 to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or

decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can

measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand 5 panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other 10 cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic 15 analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor 20 molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

25 Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate

the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The

biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of 5 such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and ^3H thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound 10 stimulates proliferation by determining the uptake of ^3H thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of ^3H thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor 15 for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a 20 second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a 25 particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or

enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

10

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

15

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

20

25 In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention

(e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the

polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells 5 which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other 10 agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is 15 separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for 20 compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known 25 in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned

drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained in the cDNA plasmid:Z identified in Table 1. In one embodiment, antisense sequence is generated internally, by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J., Neurochem. 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were

performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5' end and a HindIII site on the 3' end.

- 5 Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl₂, 10MM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the polypeptide of
10 the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

15 In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to
20 produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding the polypeptide of the present invention or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can
25 be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature 29:304-310 (1981), the promoter contained

in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

5 The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of the present invention. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case 10 of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a 15 tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

 Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' 20 untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of polynucleotide sequences described herein could be used in an antisense approach to inhibit 25 translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of

translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of mRNA of the present invention, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10
5 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may
10 include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988),
15 hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which
20 is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,
25 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-

D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-5-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; 5 Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. 10 The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of SEQ ID NO:X. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA; i.e., to increase efficiency and minimize the 15 intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express polypeptides of the present invention *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described 20 above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower 25 intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

5 The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue
10 during wound healing.

The antagonist/agonist may also be employed to treat the diseases described herein.
Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a)
15 an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

Other Activities

A polypeptide, polynucleotide, agonist, or antagonist of the present invention, as a
20 result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

25 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and

ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may 5 also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and 10 aid in tissue transplants or bone grafts.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for preventing hair loss, since FGF family members activate hair-forming 15 cells and promotes melanocyte growth. Along the same lines, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may 20 also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may 25 also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence

of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or cDNA plasmid:Z.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions 5 identified for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or cDNA plasmid:Z.

10 Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or cDNA plasmid:Z.

15 A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X in the range of positions identified for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or cDNA plasmid:Z.

20 Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto and/or cDNA plasmid:Z, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of 25 only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises cDNA plasmid:Z.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the 5 nucleotide sequence of cDNA plasmid:Z.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of an open reading frame sequence encoded by cDNA plasmid:Z.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence 10 which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by cDNA plasmid:Z.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by cDNA plasmid:Z.

15 A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by cDNA plasmid:Z.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a 20 sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto and a nucleotide sequence encoded by cDNA plasmid:Z; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid 25 molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto and a nucleotide sequence encoded by cDNA plasmid:Z.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto or cDNA plasmid:Z which encodes a protein, wherein the method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto and a nucleotide sequence of cDNA plasmid:Z.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

5 Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand
10 thereto and a nucleotide sequence encoded by cDNA plasmid:Z. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary
15 strand thereto and/or a polypeptide encoded by cDNA plasmid:Z.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and/or a polypeptide encoded by cDNA plasmid:Z.

20 Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and/or a polypeptide encoded by cDNA plasmid:Z.

Further preferred is an isolated polypeptide comprising an amino acid sequence at
25 least 95% identical to the complete amino acid sequence of SEQ ID NO:Y; a polypeptide

encoded by SEQ ID NO:X or the complementary strand thereto and/or a polypeptide encoded by cDNA plasmid:Z.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete 5 amino acid sequence of a polypeptide encoded by cDNA plasmid:Z

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a portion of said polypeptide encoded by cDNA plasmid:Z; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and/or the polypeptide sequence of SEQ ID NO:Y.

10 Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of a polypeptide encoded by cDNA plasmid:Z.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid 15 sequence of a polypeptide encoded by cDNA plasmid:Z.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of a polypeptide encoded by cDNA plasmid:Z.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 20 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and a polypeptide encoded by cDNA plasmid:Z.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 25 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary

strand thereto and a polypeptide encoded by cDNA plasmid:Z; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 5 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid 10 sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and a polypeptide encoded by cDNA plasmid:Z.

Also preferred is the above method wherein said step of comparing sequences is 15 performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence 20 of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and a polypeptide encoded by cDNA plasmid:Z.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules 25 comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein

at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleic acid sequence identified in

- 5 Table I encoding a polypeptide, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide
10 encoded by SEQ ID NO:X or the complementary strand thereto and a polypeptide encoded by cDNA plasmid:Z.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

- Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence
15 which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and a polypeptide encoded by cDNA plasmid:Z.

- 20 Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

- Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: polypeptide
25 sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and a polypeptide encoded by cDNA plasmid:Z.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the 5 recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a 10 human protein comprising an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto and a polypeptide encoded by cDNA plasmid:Z. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level 15 of a protein activity, which method comprises administering to such an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to increase the level of said protein activity in said individual.

Also preferred is a method of treatment of an individual in need of a decreased level 20 of a protein activity, which method comprised administering to such an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to decrease the level of said protein activity in said individual.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth 25 column of Table 2, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 2

and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 2. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 2. In no 5 way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

Table 2

| Gene No. | cDNA Clone ID | NT SEQ ID NO: X | Contig ID | Public Accession Numbers |
|----------|---------------|-----------------|-----------|--|
| 1 | HNTSY52 | 2 | 884215 | |
| 2 | HDPSM48 | 3 | 891330 | |
| 3 | HMTBH67 | 4 | 891032 | |
| 4 | HDPGX45 | 5 | 900539 | |
| 5 | HE9OS42 | 6 | 884221 | |
| 6 | HTEMH65 | 7 | 904829 | |
| 7 | HE9PQ20 | 8 | 874181 | |
| 8 | HFIHY04 | 9 | 892572 | T74506, R12506, R35621, R60241, R60476, N24110, N31406, N38943, W15434, W39599, AA213887, AA594583, AA809324, AA829294, AA830542, AA837273, AA292128, AI089506, Z43152, F12403, AI367273, AI568309, AI149420, AI587371 |
| 9 | HWAAP61 | 10 | 889414 | |
| 10 | HMELC05 | 11 | 877842 | |
| 11 | HTXPX58 | 12 | 892096 | R01634, R01633, AA582672, AA765938, AA862772, AA706818, AI471283, AI573185, AI334217, AI351962 |
| 12 | HLDAE46 | 13 | 877556 | |

10

Table 3

| Clone ID NO: Z | Library Code |
|-------------------|---|
| HNTSY52 | H0023 H0032 H0038 H0039 H0042 H0051 H0083 H0090 H0171 H0196 H0251 H0266 H0329 H0373 H0412 H0423 H0477 H0478 H0509 H0521 H0543 H0547 H0553 H0575 H0580 H0581 H0591 H0595 H0644 H0659 |

| | |
|---------|--|
| | H0667 L0471 L0485 L0512 L0588 L0608 L0637 L0646 L0647 L0650 L0653 L0659 L0661 L0662 L0663 L0666 L0731 L0740 L0745 L0750 L0754 L0755 L0756 L0757 L0759 L0761 L0763 L0765 L0766 L0770 L0771 L0803 L0806 S0003 S0026 S0028 S0048 S0112 S0126 S0134 S0152 S0192 S0242 S0250 S0260 S0282 S0328 S0354 S0356 S0358 S0360 S0374 S0376 S0422 S0424 T0041 T0067 T0115 |
| HDPSM48 | H0031 H0171 H0373 H0427 H0494 H0521 H0580 H0599 H0638 H0687 L0375 L0471 L0485 L0517 L0596 L0649 L0663 L0731 L0748 L0777 L0779 L0804 L0806 S0126 S0206 S0212 S0356 S0366 T0110 |
| HMTBH67 | H0074 H0090 H0111 H0144 H0170 H0265 H0309 H0316 H0341 H0423 H0457 H0486 H0497 H0518 H0519 H0543 H0547 H0550 H0556 H0574 H0580 H0596 H0634 H0650 H0667 H0672 L0040 L0526 L0591 L0598 L0605 L0653 L0666 L0667 L0748 L0750 L0752 L0754 L0759 L0766 L0774 L0775 L0792 L0803 S0013 S0026 S0150 S0152 S0282 S0422 T0041 T0110 |
| HDPGX45 | H0024 H0179 H0264 H0271 H0445 H0509 H0519 H0521 H0555 H0690 L0177 L0593 L0595 L0751 L0768 L0773 L0776 L0789 L0803 L0805 S0428 T0006 |
| HE9OS42 | H0013 H0038 H0040 H0052 H0144 H0261 H0264 H0266 H0340 H0341 H0413 H0445 H0492 H0529 H0555 H0561 H0591 H0598 H0606 H0624 L0055 L0163 L0367 L0438 L0439 L0455 L0596 L0645 L0663 L0666 L0731 L0744 L0747 L0748 L0754 L0758 L0770 L0777 L0779 L0790 L0794 L0803 S0027 S0152 S0376 S0428 T0041 T0049 |
| HTEMH65 | H0255 H0327 H0616 L0438 L0439 L0595 L0659 L0741 L0749 L0756 L0758 L0769 L0770 L0779 L0783 L0809 S0002 S0051 S0150 |
| HE9PQ20 | H0024 H0038 H0123 H0144 H0265 H0309 H0364 H0399 H0412 H0486 H0529 H0553 H0580 H0599 H0632 L0363 L0439 L0471 L0558 L0598 L0649 L0659 L0662 L0743 L0748 L0757 L0758 L0775 L0777 L0779 L0792 L0803 L0805 S0002 S0003 S0027 S0028 S0032 S0042 S0206 S0358 S0374 S3012 S3014 |
| HFIHY04 | H0013 H0039 H0090 H0098 H0144 H0266 H0411 H0422 H0521 H0522 H0529 H0542 H0543 H0560 H0623 H0638 H0682 L0372 L0438 L0439 L0598 L0655 L0659 L0663 L0665 L0666 L0731 L0740 L0747 L0749 L0752 L0755 L0756 L0761 L0766 L0768 L0772 L0777 L0779 L0803 S0003 S0116 S0152 S0194 S0282 S0358 T0042 T0071 |
| HWAAP61 | H0036 H0038 H0050 H0083 H0124 H0144 H0264 H0265 H0271 H0288 H0318 H0352 H0427 H0510 H0521 H0543 H0555 H0556 H0581 H0586 H0597 H0598 H0616 H0657 L0361 L0364 L0527 L0592 L0596 L0644 L0655 L0657 L0659 L0662 L0664 L0666 L0731 L0747 L0748 L0754 |

| | |
|---------|---|
| | L0758 L0759 L0764 L0766 L0770 L0777 L0789 L0794 L0803 L0804 L0805 L0809 S0010 S0026 S0032 S0116 S0126 S0328 S0344 S0354 S0356 S0358 S0420 |
| HMLEC05 | H0135 H0266 H0299 H0542 H0674 L0352 L0438 L0439 L0528 L0592 L0608 L0663 L0740 L0748 L0749 L0750 L0758 S0420 |
| HTXPX58 | H0222 H0290 H0356 H0375 H0412 H0439 H0497 H0518 H0581 H0591 H0596 H0622 H0687 L0375 L0646 L0659 L0748 L0749 L0766 L0768 L0777 S0002 S0037 S0132 S0214 S0374 T0110 |
| HLDAE46 | H0509 L0809 |

Table 4

| SEQ ID NO: X | Cytologic Band or Chromosome: | OMIM ID: |
|-----------------|----------------------------------|---------------|
| 2 | Chromosome 1 | |
| 4 | Chromosome X | |
| 5 | Chromosome 1 | |
| 7 | Chromosome 10 | |
| 8 | Chromosome 13 | |
| 9 | Chromosome 12 | |
| 10 | Chromosome 17 | |
| 12 | Chromosome 16 | |
| 13 | 8q22 | 216550 259730 |

5 Table 5

| Library Code | Library Description |
|-----------------|-----------------------------|
| H0013 | Human 8 Week Whole Embryo |
| H0023 | Human fetal lung |
| H0024 | Human Fetal Lung III |
| H0031 | Human Placenta |
| H0032 | Human Prostate |
| H0036 | Human Adult Small Intestine |
| H0038 | Human Testes |
| H0039 | Human Pancreas Tumor |
| H0040 | Human Testes Tumor |
| H0042 | Human Adult Pulmonary |

| | |
|-------|---|
| H0050 | Human Fetal Heart |
| H0051 | Human Hippocampus |
| H0052 | Human Cerebellum |
| H0074 | Human Platelets |
| H0083 | HUMAN JURKAT MEMBRANE BOUND POLYSOMES |
| H0090 | Human T-Cell Lymphoma |
| H0098 | Human Adult Liver, subtracted |
| H0111 | Human Placenta, subtracted |
| H0123 | Human Fetal Dura Mater |
| H0124 | Human Rhabdomyosarcoma |
| H0135 | Human Synovial Sarcoma |
| H0144 | Nine Week Old Early Stage Human |
| H0170 | 12 Week Old Early Stage Human |
| H0171 | 12 Week Old Early Stage Human, II |
| H0179 | Human Neutrophil |
| H0196 | Human Cardiomyopathy, subtracted |
| H0222 | Activated T-Cells, 8 hrs, subtracted |
| H0251 | Human Chondrosarcoma |
| H0255 | breast lymph node CDNA library |
| H0261 | H. cerebellum, Enzyme subtracted |
| H0264 | human tonsils |
| H0265 | Activated T-Cell (12hs)/Thiouridine labelledEco |
| H0266 | Human Microvascular Endothelial Cells, fract. A |
| H0271 | Human Neutrophil, Activated |
| H0288 | Human OB HOS control fraction I |
| H0290 | Human OB HOS treated (1 nM E2) fraction I |
| H0299 | HCBA's differential consolidation |
| H0309 | Human Chronic Synovitis |
| H0316 | HUMAN STOMACH |
| H0318 | HUMAN B CELL LYMPHOMA |
| H0327 | human corpus colosum |
| H0329 | Dermatofibrosarcoma Protuberance |
| H0340 | Corpus Callosum |
| H0341 | Bone Marrow Cell Line (RS4,11) |
| H0352 | wilm's tumor |

| | |
|-------|--|
| H0356 | Human Kidney |
| H0364 | Human Osteoclastoma, excised |
| H0373 | Human Heart |
| H0375 | Human Lung |
| H0399 | Human Kidney Cortex, re-rescue |
| H0411 | H Female Bladder, Adult |
| H0412 | Human umbilical vein endothelial cells, IL-4 induced |
| H0413 | Human Umbilical Vein Endothelial Cells, uninduced |
| H0422 | T-Cell PHA 16 hrs |
| H0423 | T-Cell PHA 24 hrs |
| H0427 | Human Adipose |
| H0439 | Human Eosinophils |
| H0445 | Spleen, Chronic lymphocytic leukemia |
| H0457 | Human Eosinophils |
| H0477 | Human Tonsil, Lib 3 |
| H0478 | Salivary Gland, Lib 2 |
| H0486 | Hodgkin's Lymphoma II |
| H0492 | HL-60, RA 4h, Subtracted |
| H0494 | Keratinocyte |
| H0497 | HEL cell line |
| H0509 | Liver, Hepatoma |
| H0510 | Human Liver, normal |
| H0518 | pBMC stimulated w/ poly I/C |
| H0519 | NTERA2, control |
| H0521 | Primary Dendritic Cells, lib 1 |
| H0522 | Primary Dendritic cells,frac 2 |
| H0529 | Myeloid Progenitor Cell Line |
| H0542 | T Cell helper I |
| H0543 | T cell helper II |
| H0547 | NTERA2 teratocarcinoma cell line+retinoic acid (14 days) |
| H0550 | H. Epididymus, cauda |
| H0553 | Human Placenta |
| H0555 | Rejected Kidney, lib 4 |
| H0556 | Activated T-cell(12h)/Thiouridine-re-excision |
| H0560 | KMH2 |

| | |
|-------|---|
| H0561 | L428 |
| H0574 | Hepatocellular Tumor, re-excision |
| H0575 | Human Adult Pulmonary,re-excision |
| H0580 | Dendritic cells, pooled |
| H0581 | Human Bone Marrow, treated |
| H0586 | Healing groin wound, 6.5 hours post incision |
| H0591 | Human T-cell lymphoma,re-excision |
| H0595 | Stomach cancer (human),re-excision |
| H0596 | Human Colon Cancer,re-excision |
| H0597 | Human Colon, re-excision |
| H0598 | Human Stomach,re-excision |
| H0599 | Human Adult Heart,re-excision |
| H0606 | Human Primary Breast Cancer,re-excision |
| H0616 | Human Testes, Reexcision |
| H0622 | Human Pancreas Tumor, Reexcision |
| H0623 | Human Umbilical Vein, Reexcision |
| H0624 | 12 Week Early Stage Human II, Reexcision |
| H0632 | Hepatocellular Tumor,re-excision |
| H0634 | Human Testes Tumor, re-excision |
| H0638 | CD40 activated monocyte dendridic cells |
| H0644 | Human Placenta (re-excision) |
| H0650 | B-Cells |
| H0657 | B-cells (stimulated) |
| H0659 | Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma |
| H0667 | Stromal cells(HBM3.18) |
| H0672 | Ovary, Cancer: (4004576 A8) |
| H0674 | Human Prostate Cancer, Stage C, re-excision |
| H0682 | Ovarian cancer, Serous Papillary Adenocarcinoma |
| H0687 | Human normal ovary(#9610G215) |
| H0690 | Ovarian Cancer, # 9702G001 |
| L0040 | Human colon mucosa |
| L0055 | Human promyelocyte |
| L0163 | Human heart cDNA (Y Nakamura) |
| L0177 | Human newborn melanocytes (T. Vogt) |
| L0352 | Normalized infant brain, Bento Soares |
| L0361 | Stratagene ovary (#937217) |

| | |
|-------|---|
| L0363 | NCI_CGAP_GC2 |
| L0364 | NCI_CGAP_GC5 |
| L0367 | NCI_CGAP_Sch1 |
| L0372 | NCI_CGAP_Co12 |
| L0375 | NCI_CGAP_Kid6 |
| L0438 | normalized infant brain cDNA |
| L0439 | Soares infant brain 1NIB |
| L0455 | Human retina cDNA randomly primed sublibrary |
| L0471 | Human fetal heart, Lambda ZAP Express |
| L0485 | STRATAGENE Human skeletal muscle cDNA library, cat. #936215. |
| L0512 | NCI_CGAP_Ov36 |
| L0517 | NCI_CGAP_Pr1 |
| L0526 | NCI_CGAP_Pr12 |
| L0527 | NCI_CGAP_Ov2 |
| L0528 | NCI_CGAP_Pr5 |
| L0558 | NCI_CGAP_Ov40 |
| L0588 | Stratagene endothelial cell 937223 |
| L0591 | Stratagene HeLa cell s3 937216 |
| L0592 | Stratagene hNT neuron (#937233) |
| L0593 | Stratagene neuroepithelium (#937231) |
| L0595 | Stratagene NT2 neuronal precursor 937230 |
| L0596 | Stratagene colon (#937204) |
| L0598 | Morton Fetal Cochlea |
| L0605 | Stratagene fetal spleen (#937205) |
| L0608 | Stratagene lung carcinoma 937218 |
| L0637 | NCI_CGAP_Brn53 |
| L0644 | NCI_CGAP_Co20 |
| L0645 | NCI_CGAP_Co21 |
| L0646 | NCI_CGAP_Co14 |
| L0647 | NCI_CGAP_Sar4 |
| L0649 | NCI_CGAP_GU1 |
| L0650 | NCI_CGAP_Kid13 |
| L0653 | NCI_CGAP_Lu28 |
| L0655 | NCI_CGAP_Lym12 |
| L0657 | NCI_CGAP_Ov23 |
| L0659 | NCI_CGAP_Pan1 |

| | |
|-------|--------------------------------------|
| L0661 | NCI_CGAP_Mel15 |
| L0662 | NCI_CGAP_Gas4 |
| L0663 | NCI_CGAP_Ut2 |
| L0664 | NCI_CGAP_Ut3 |
| L0665 | NCI_CGAP_Ut4 |
| L0666 | NCI_CGAP_Ut1 |
| L0667 | NCI_CGAP_CML1 |
| L0731 | Soares_pregnant_uterus_NbHPU |
| L0740 | Soares melanocyte 2NbHM |
| L0741 | Soares adult brain N2b4HB55Y |
| L0743 | Soares breast 2NbHBst |
| L0744 | Soares breast 3NbHBst |
| L0745 | Soares retina N2b4HR |
| L0747 | Soares_fetal_heart_NbHH19W |
| L0748 | Soares fetal liver spleen 1NFLS |
| L0749 | Soares_fetal_liver_spleen_1NFLS_S1 |
| L0750 | Soares_fetal_lung_NbHL19W |
| L0751 | Soares ovary tumor NbHOT |
| L0752 | Soares_parathyroid_tumor_NbHPA |
| L0754 | Soares placenta Nb2HP |
| L0755 | Soares_placenta_8to9weeks_2NbHP8to9W |
| L0756 | Soares_multiple_sclerosis_2NbHMSP |
| L0757 | Soares_senescent_fibroblasts_NbHSF |
| L0758 | Soares_testis_NHT |
| L0759 | Soares_total_fetus_Nb2HF8_9w |
| L0761 | NCI_CGAP CLL1 |
| L0763 | NCI_CGAP_Br2 |
| L0764 | NCI_CGAP_Co3 |
| L0765 | NCI_CGAP_Co4 |
| L0766 | NCI_CGAP_GCB1 |
| L0768 | NCI_CGAP_GC4 |
| L0769 | NCI_CGAP_Brn25 |
| L0770 | NCI_CGAP_Brn23 |
| L0771 | NCI_CGAP_Co8 |
| L0772 | NCI_CGAP_Co10 |
| L0773 | NCI_CGAP_Co9 |
| L0774 | NCI_CGAP_Kid3 |
| L0775 | NCI_CGAP_Kid5 |

| | |
|-------|---|
| L0776 | NCI_CGAP_Lu5 |
| L0777 | Soares_NhHMPu_S1 |
| L0779 | SoaresNFL_T_GBC_S1 |
| L0783 | NCI_CGAP_Pr22 |
| L0789 | NCI_CGAP_Sub3 |
| L0790 | NCI_CGAP_Sub4 |
| L0792 | NCI_CGAP_Sub6 |
| L0794 | NCI_CGAP_GC6 |
| L0803 | NCI_CGAP_Kid11 |
| L0804 | NCI_CGAP_Kid12 |
| L0805 | NCI_CGAP_Lu24 |
| L0806 | NCI_CGAP_Lu19 |
| L0809 | NCI_CGAP_Pr28 |
| S0002 | Monocyte activated |
| S0003 | Human Osteoclastoma |
| S0010 | Human Amygdala |
| S0013 | Prostate |
| S0026 | Stromal cell TF274 |
| S0027 | Smooth muscle, serum treated |
| S0028 | Smooth muscle, control |
| S0032 | Smooth muscle-ILb induced |
| S0037 | Smooth muscle, IL1b induced |
| S0042 | Testes |
| S0048 | Human Hypothalamus, Alzheimer's |
| S0051 | Human Hypothalamus, Schizophrenia |
| S0112 | Hypothalamus |
| S0116 | Bone marrow |
| S0126 | Osteoblasts |
| S0132 | Epithelial-TNF α and INF induced |
| S0134 | Apoptotic T-cell |
| S0150 | LNCAP prostate cell line |
| S0152 | PC3 Prostate cell line |
| S0192 | Synovial Fibroblasts (control) |
| S0194 | Synovial hypoxia |
| S0206 | Smooth Muscle- HASTE normalized |
| S0212 | Bone Marrow Stromal Cell, untreated |
| S0214 | Human Osteoclastoma, re-excision |
| S0242 | Synovial Fibroblasts (IL1/TNF), subt |

| | |
|-------|--|
| S0250 | Human Osteoblasts II |
| S0260 | Spinal Cord, re-excision |
| S0282 | Brain Frontal Cortex, re-excision |
| S0328 | Palate carcinoma |
| S0344 | Macrophage-oxLDL, re-excision |
| S0354 | Colon Normal II |
| S0356 | Colon Carcinoma |
| S0358 | Colon Normal III |
| S0360 | Colon Tumor II |
| S0366 | Human Soleus |
| S0374 | Normal colon |
| S0376 | Colon Tumor |
| S0420 | CHME Cell Line,untreated |
| S0422 | Mo7e Cell Line GM-CSF treated (1ng/ml) |
| S0424 | TF-1 Cell Line GM-CSF Treated |
| S0428 | Neutrophils control, re-excision |
| S3012 | Smooth Muscle Serum Treated, Norm |
| S3014 | Smooth muscle, serum induced,re-exc |
| T0006 | Human Pineal Gland |
| T0041 | Jurkat T-cell G1 phase |
| T0042 | Jurkat T-Cell, S phase |
| T0049 | Aorta endothelial cells + TNF-a |
| T0067 | Human Thyroid |
| T0071 | Human Bone Marrow |
| T0110 | Human colon carcinoma (HCC) cell line, remake |
| T0115 | Human Colon Carcinoma (HCC) cell line |

Table 6

| OMIM ID | OMIM Description |
|---------|---|
| 216550 | Cohen syndrome (2) |
| 259730 | Renal tubular acidosis-osteopetrosis syndrome (3) |

5 Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

*Examples*Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

5

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

| | <u>Vector Used to Construct Library</u> | <u>Corresponding Deposited Plasmid</u> |
|----|---|--|
| | Lambda Zap | pBluescript (pBS) |
| 15 | Uni-Zap XR | pBluescript (pBS) |
| | Zap Express | pBK |
| | Iafmid BA | plafmid BA |
| | pSport1 | pSport1 |
| | pCMVSport 2.0 | pCMVSport 2.0 |
| 20 | pCMVSport 3.0 | pCMVSport 3.0 |
| | pCR®2.1 | pCR®2.1 |

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short et al., *Nucleic Acids Res.*, 16:7583-7600 (1988); Alting-Mees et al., *Nucleic Acids Res.*, 17:9494 (1989)) and pBK (Alting-Mees et al., *Strategies*, 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N.

Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which 5 flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life 10 Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR®2.1, which is 15 available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, *Nuc. Acids Res.*, 16:9677-9686 (1988) and Mead et al., *Bio/Technology*, 9 (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as 20 well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. 25 Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA

clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by 5 screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P - γ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A 10 Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a 15 density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

20 Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 μl of reaction mixture with 0.5 ug of the above cDNA 25 template. A convenient reaction mixture is 1.5-5 mM MgCl₂, 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq

polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is 5 verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a 10 method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., *Nucleic Acids Res.*, 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence 15 of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may 20 interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

25 This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template

for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

5

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

10

Example 3: Tissue Distribution of Polypeptide

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled 15 with P³² using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

20

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and the films developed according to standard procedures.

25

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute 5 cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

10

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the 15 cDNA insert should preferably contain restriction sites, such as BamHI and XbaI and initiation/stop codons, if necessary, to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^R), a bacterial origin of replication (ori), an IPTG-regulatable 20 promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) 25 which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^R). Transformants are identified by their ability to

grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture 5 is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation 10 (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step 15 procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

20 The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The 25 renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM immidazole. Immidazole is removed by a final

dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a 5 polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an *E. coli* origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, 10 Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction 15 sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

20

Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

25

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm

(Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

5 The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

10 The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

15 Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

20 To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of
25 the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 μ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

15

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides

by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as 5 the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone is amplified using the PCR protocol described in Example 1 using primers with appropriate restriction sites and 10 initiation/stop codons. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station 15 Bulletin NO: 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, 20 can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning 25 Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual

colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five μ g of a plasmid containing the polynucleotide is co-transfected with 1.0 μ g of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", 5 Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One μ g of BaculoGold™ virus DNA and 5 μ g of the plasmid are mixed in a sterile well of a microtiter plate containing 50 μ l of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μ l Lipofectin plus 90 μ l Grace's medium are added, mixed and incubated for 15 minutes at room 10 temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

15 After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by 20 Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are 25 harvested and then they are stored at 4° C.

- To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with
- 5 SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 μ Ci of 35 S-methionine and 5 μ Ci 35 S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).
- 10 Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, and pCMVSport 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and

Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt et al., *J. Biol. Chem.*, 253:1357-1370 (1978); Hamlin et al., *Biochem. et Biophys. Acta*, 1097:107-143 (1990); Page et al., *Biotechnology*, 9:64-68 (1991)). Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J.*, 227:277-279 (1991); Bebbington et al., *BioTechnology*, 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No.: 37146), the expression vectors pC4 (ATCC Accession No.: 209646) and pC6 (ATCC Accession No.: 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., *Cell*, 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol 5 outlined in Example 1 using primers with appropriate restrictions sites and initiation/stop codons, if necessary. The vector can be modified to include a heterologous signal sequence if necessary for secretion. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with 10 appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction 15 enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μ g of the expression plasmid pC6 is cotransfected with 0.5 μ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a 20 group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones 25 are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates

containing even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

5

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding 10 protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., *Nature*, 331:84-86 (1988)) The polypeptides can also be fused to heterologous polypeptide sequences to facilitate secretion and intracellular trafficking (e.g., KDEL). Moreover, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific 15 subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a 20 polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector, and initiation/stop codons, if necessary.

25 For example, if pC4 (Accession No.: 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next,

the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

5 If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

10 Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAA ACTCACACATGCCACC GTGCCAGCACCTGAATT CG
AGGGTGCACCGTCAGTCTT CCTCTTCCCCC AAAACCC AAGGACACCCTCATGATCTCCGGACTC
CTGAGGT CACATCGTGGTGGACGTAA GGCACGAAGACCC TGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGGAGGAGCAGTACAACAGCACGT
15 ACCGTGTGGTCAGCGT CCTCACCGT CCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCAAACCCCATCGAGAAA ACCATCTCCAAGCCAAGGGCAGCC
CCGAGAACCA CAGGTGTACACCCTGCCCTATCCGGGATGAGCTGACCAAGAAC CAGGTCAAGCC
TGACCTGCCTGGTCAAAGGTTCTATCCAAGCGACATGCCGTGGAGTGGAGAGCAATGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCGTGCTGGACTCCGACGGCTCCTCTCCTACAGC
20 AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAACGTCTCTCATGCTCCGTATGCATGA
GGCTCTGCACAACC ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGCACGGC
CGCGACTCTAGAGGAT (SEQ ID NO:1)

Example 10: Formulating a Polypeptide

25 The polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the secreted polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors

known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of polypeptide administered parenterally per dose will be in the range of about 1 $\mu\text{g}/\text{kg}/\text{day}$ to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the polypeptide is typically administered at a dose rate of about 1 $\mu\text{g}/\text{kg}/\text{hour}$ to about 50 $\mu\text{g}/\text{kg}/\text{hour}$, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the polypeptide of the invention are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. NO: 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and

Langer, *Chem. Tech.*, 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped polypeptides. Liposomes containing the secreted polypeptide are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. USA*, 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. USA*, 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal 10 secreted polypeptide therapy.

For parenteral administration, in one embodiment, the polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with 15 other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the 20 product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that 25 enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate,

succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The polypeptide is typically formulated in such vehicles at a concentration of about 10 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any polypeptide to be used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for 20 reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more 25 containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a

governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

5

Example 11: Method of Treating Decreased Levels of the Polypeptide

It will be appreciated that conditions caused by a decrease in the standard or normal expression level of a polypeptide in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted and/or soluble form. Thus, 10 the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 15 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 10.

Example 12: Method of Treating Increased Levels of the Polypeptide

20 Antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day 25 for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 10.

Example 13: Method of Treatment Using Gene Therapy - Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy.

- 5 The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12
10 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

- 15 pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

- The cDNA encoding a polypeptide of the present invention can be amplified using
20 PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase.
25 The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then

plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum

- 5 (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is
10 harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then
15 virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after
20 having been grown to confluence on cytodex 3 microcarrier beads.

Example 14: Gene Therapy Using Endogenous TGF-beta Receptor Genes

Another method of gene therapy according to the present invention involves operably associating the endogenous TGF-beta receptor gene sequence with a promoter via
25 homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996;

International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

5 Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of the endogenous TGF-beta receptor gene, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of TGF-beta receptor gene so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be
10 amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

15 The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by
20 phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

25 Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous TGF-beta receptor gene

sequence. This results in the expression of TGF-beta receptor in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase 5 fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells 10 resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10⁶ cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the TGF-beta receptor locus, plasmid pUC18 (MBI 15 Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two TGF-beta receptor non-coding gene sequences are amplified via PCR: one TGF-beta receptor non-coding sequence (TGF-beta receptor fragment 1) is amplified with a HindIII site at the 5' end and an XbaI site at the 3'end; the other TGF-beta receptor non-coding sequence (TGF-beta receptor fragment 20 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and TGF-beta receptor fragments are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; TGF-beta receptor fragment 1 - XbaI; TGF-beta receptor fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

25 Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension

(containing approximately 1.5.X10⁶ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μ F and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving
5 cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a
10 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.
15

Example 15: Method of Treatment Using Gene Therapy - In Vivo

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) TGF-beta receptor sequences
20 into an animal to increase or decrease the expression of the TGF-beta receptor polypeptide. The TGF-beta receptor polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of the TGF-beta receptor polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO: 5693622, 5705151,
25 5580859; Tabata et al., *Cardiovasc. Res.* 35(3):470-479 (1997), Chao J et al., *Pharmacol. Res.*, 35(6):517-522 (1997), Wolff, *Neuromuscul. Disord.* 7(5):314-318 (1997), Schwartz et

al., *Gene Ther.*, 3(5):405-411 (1996), Tsurumi Y. et al., *Circulation*, 94(12):3281-3290 (1996) (incorporated herein by reference).

The TGF-beta receptor polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the 5 interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The TGF-beta receptor polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, 10 including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the TGF-beta receptor polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner et al., *Ann. NY Acad. Sci.*, 772:126-139 (1995) and Abdallah et al., *Biol. Cell*, 85(1):1-7 (1995)) which can be prepared by methods well known to those skilled in the art.

15 The TGF-beta receptor polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells 20 is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide constructs can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, 25 thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue.

Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked TGF-beta receptor polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked TGF-beta receptor polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected TGF-beta receptor polynucleotide in muscle *in vivo* is determined as follows. Suitable TGF-beta receptor template DNA for production of

mRNA coding for TGF-beta receptor polypeptide is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

5 Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The TGF-beta receptor template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2
10 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for TGF-beta receptor protein expression. A time course
15 for TGF-beta receptor protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of TGF-beta receptor DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages
20 and other treatment parameters in humans and other animals using TGF-beta receptor naked DNA.

Example 16: Production of an Antibody

a) Hybridoma Technology
25 The antibodies of the present invention can be prepared by a variety of methods.
(See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing

TGF-beta receptor polypeptide(s) are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of TGF-beta receptor polypeptide(s) is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce 5 polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for TGF-beta receptor polypeptide(s) are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In 10 general, an animal (preferably a mouse) is immunized with TGF-beta receptor polypeptide(s) or, more preferably, with a secreted TGF-beta receptor polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 15 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in 20 HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the TGF-beta receptor polypeptide(s).

Alternatively, additional antibodies capable of binding to TGF-beta receptor 25 polypeptide(s) can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is

possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the TGF-beta receptor protein-specific antibody can be blocked by TGF-beta receptor polypeptide(s). Such antibodies comprise anti-idiotypic antibodies to the TGF-beta receptor protein-specific antibody and are used to immunize an animal to induce formation of further TGF-beta receptor protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulian et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed Against TGF-beta receptor Polypeptide(s) From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against TGF-beta receptor polypeptide(s) to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library.

A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10⁹ E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with 5 shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10⁸ TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin 10 and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III 15 protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations 20 (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10¹³ transducing units/ml (ampicillin-resistant clones).

Panning of the Library.

25 Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for

2 hours at 37°C and then washed 3 times in PBS. Approximately 10¹³ TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders.

15 Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by 20 sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

25 **Example 17: Generation of a chimeric TGF-beta receptor protein receptors**

A chimeric receptor is generated following the protocol of Luo et al., by fusing the nucleotides encoding for the extracellular domain of a receptor of interest to the nucleotides encoding for the transmembrane domain and intracellular domain of the TGF-beta receptor protein of the present invention (Luo, K., et al., *EMBO Journal*, 15:4485-96 (1996), which is 5 herein incorporated by reference in its entirety). Alternatively, the chimeric receptor could be generated using the extracellular domain of the TGF-beta receptor protein of the present invention fused to the transmembrane and intracellular domains of a receptor of interest, such that binding of TGF-beta to the chimeric receptor could be detected. Clearly, many combinations of domains from heterologous transmembrane receptor proteins could be fused 10 using methods known in the art to produce a chimeric receptor protein.

The resulting DNA encoding for the chimeric receptor is cloned into an appropriate mammalian, baculoviral, viral, or bacterial expression vector, such as, for example, pC4, pCDNA3, or pA2, as discussed supra. Mammalian host cells that could be used for expression of the chimeric receptor include the pre-B cell line BaF3.

15

Example 18: Detection of Expression of the Chimeric EpoR/TGF-beta Receptor Protein on the Plasma Membrane by Measurement of Epo Binding

Detection of the expression of TGF-beta chimeric receptors can be achieved using the detection assay disclosed by Beutler et al. in U.S. Patent 5,925,548, which is herein 20 incorporated by reference in its entirety. Briefly, radiolabeled (^{125}I) ligand which binds extracellular domain of the chimeric receptor of interest is obtained. 2×10^6 COS cells are plated in a 10 cm plate, transfected according to a CaPO₄ method (Chen & Okayama, 1987), using 20 µg of total DNA purified by the Quiagen technique. Transfections are performed in duplicate plates, from which the cells are pooled after 12 hours. The cells are then split into 25 triplicate wells of a six-well plate at a density of 1×10^6 per well. After five hours allowed for adherence to the plastic, cells are incubated on ice in 1 ml of medium containing 2% serum

and 2% antibiotic mixture (pen/strep/GIBCO), 25 mM HEPES buffer, and 0.525 pM iodinated Epo (415 nCi/ml). Incubation is allowed to continue for 2 hours with occasional gentle shaking by hand (on ice). 3 ml of ice-cold PBS containing 1 mM EDTA is then added to each well. The cells are harvested by trituration, pelleted, transferred to Eppendorf tube(s),
5 and washed once more with PBS/EDTA solution (all procedures are carried out in the cold). The cell pellets are then counted for bound radioactivity.

Example 19: In vitro kinase assay

The following assay is designed to detect signal transduction activity of the TGF-beta receptor polypeptides of the present invention, based upon the *in vitro* kinase assay of Luo, K., et al., *EMBO Journal*, 15:4485-96 (1996), which is herein incorporated by reference in its entirety. More specifically, about 3×10^8 Ba/F3 cells, or other appropriate cell line, expressing the TGF-beta chimeric receptors, where the chimeric receptor comprises the intracellular domain of a TGF-beta receptor of the present invention, are washed, stimulated with fresh media (RPMI medium supplemented with 10% fetal bovine serum (FBS), and 10% WEHI-3 cell-conditioned medium) with or without an appropriate amount of ligand or activating antibody for 10 min at room temperature, and lysed with NP-40 lysis buffer (1% NP-40, 50mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10 μ g/ml aprotinin and 2 mM phenylmethylsulfonyl fluoride (PMSF).) The TGF-beta chimeric receptor is isolated by
15 immunoprecipitation using antibodies to the cytoplasmic domains of the TGF-beta receptors.
In vitro phosphorylation reactions using the immunoprecipitates of the chimeric receptors are conducted in 20 μ l of kinase buffer (50 mM Tris, pH 7.5, 10mM MgCl₂) in the presence of 10 μ Ci [γ -³²P] ATP (3000 Ci/nmol) for 10 min at 30°C. The reaction is terminated by the addition of 5 mM EDTA, and the immune complexes are eluted from the beads by boiling.
20 The labeled chimeric receptors are then re-immunoprecipitated with antibodies to the extracellular domain of the TGF-beta chimeric receptor. Phosphorylation is observed by
25

SDS-Page and autoradiography. The extracellular portion of the TGF-beta chimeric receptor may be derived from any appropriate receptor known in the art.

Example 20: Identification of TGF-beta receptor protein-Interacting Proteins

5 The TGF-beta polypeptides of the invention are research tools for the identification, characterization and purification of interacting Smad proteins or TGF-beta receptor proteins, such as T β R-I, for example, or other signal transduction pathway proteins. Briefly, labeled TGF-beta receptor protein is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, membrane-bound TGF-beta receptor
10 protein is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as colon cancer cells, is passed over the column, and molecules with appropriate affinity bind to the TGF-beta receptor protein. The TGF-beta receptor protein-complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify
15 the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 21: Kinase Assay for Receptor Protein-Interacting Proteins

 To determine which candidate TGF-beta receptor protein-interacting proteins may be
20 directly involved in signal transduction, a modified kinase assay as described by Hirai et al. may be employed (Hirai, S., et al., *J. Biol. Chem.*, 272:15167-73 (1997), which is herein incorporated by reference in its entirety). Briefly, isolated TGF-beta receptor proteins of the present invention are fixed on 2 μ l of protein G-Sepharose and suspended in 20 μ l of assay mixture containing 20 mM HEPES, pH 7.5, 15 mM MgCl₂, 15 mM -glycerophosphate, 0.1 mM Na₃VO₄, 2 mM dithiothreitol, 25 μ M ATP, 5 μ Ci of [-32P]ATP, and 1 μ g of each
25 candidate TGF-beta receptor protein-interacting protein, as well as with appropriate controls.

The kinase reaction is carried out at 30 °C for 20 min., and stopped by adding 25 µl of SDS sample buffer. The phosphorylation of each candidate receptor protein-interacting protein is detected by SDS-PAGE and autoradiograph, and the incorporation of ³²P by candidate receptor protein-interacting proteins is indicative of phosphorylation by TGF-beta receptor 5 proteins.

The TGF-beta receptors of the present invention were disclosed in U.S. provisional application serial numbers 60/148,682; and 60/154,887, each of which are herein incorporated by reference in their entirety.

10 It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

15 The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties.

| | | | |
|--|----------|-------------------------------|------------|
| Applicant's or agent's file reference number | PT014PCT | International application No. | UNASSIGNED |
|--|----------|-------------------------------|------------|

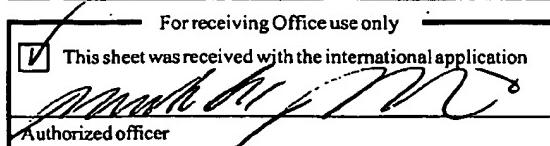
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

| | | |
|--|------------------|--|
| A. The indications made below relate to the microorganism referred to in the description on page <u>44</u> , line <u>N/A</u> | | |
| B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/> | | |
| Name of depositary institution American Type Culture Collection | | |
| Address of depositary institution (<i>including postal code and country</i>) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America | | |
| Date of deposit | Accession Number | |
| 13 August 1999 | PTA-539 | |
| C. ADDITIONAL INDICATIONS (<i>leave blank if not applicable</i>) This information is continued on an additional sheet <input type="checkbox"/> | | |
| | | |
| D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (<i>if the indications are not for all designated States</i>) | | |
| Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3 | | |
| E. SEPARATE FURNISHING OF INDICATIONS (<i>leave blank if not applicable</i>) | | |
| The indications listed below will be submitted to the International Bureau later (<i>specify the general nature of the indications e.g., "Accession Number of Deposit"</i>) | | |

For receiving Office use only

This sheet was received with the international application



Authorized officer

For International Bureau use only

This sheet was received by the International Bureau on:

Authorized officer

ATCC Deposit No. PTA-539**Page No. 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-539

Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:
 - (a) the polynucleotide shown as SEQ ID NO:X or the polynucleotide encoded by a cDNA included in ATCC Deposit No:Z;
 - (b) a polynucleotide encoding a biologically active polypeptide fragment of SEQ ID NO:Y or a biologically active polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z;
 - (c) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z;
 - (d) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(c), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.
2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide comprises a nucleotide sequence encoding a soluble polypeptide.
3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide comprises the entire nucleotide sequence of SEQ ID NO:X or a cDNA included in ATCC Deposit No:Z..

5. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide is DNA.

6. The isolated nucleic acid molecule of claim 3, wherein the polynucleotide is RNA.

7. A vector comprising the isolated nucleic acid molecule of claim 1.

8. A host cell comprising the vector of claim 7.

9. A recombinant host cell comprising the nucleic acid molecule of claim 1 operably limited to a heterologous regulating element which controls gene expression.

10. A method of producing a polypeptide comprising expressing the encoded polypeptide from the host cell of claim 9 and recovering said polypeptide.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:
(a) the polypeptide shown as SEQ ID NO:Y or the polypeptide encoded by the cDNA;

- (b) a polypeptide fragment of SEQ ID NO:Y or the polypeptide encoded by the cDNA;
- (c) a polypeptide epitope of SEQ ID NO:Y or the polypeptide encoded by the cDNA; and
- (d) a variant of SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, comprising a polypeptide having SEQ ID NO:Y.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:
(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and
(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

- (a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and
- (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

- (a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and
- (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

- (a) contacting the polypeptide of claim 11 with a binding partner; and
- (b) determining whether the binding partner effects an activity of the polypeptide.

21. A method of screening for molecules which modify activities of the polypeptide of claim 11 comprising:

- (a) contacting said polypeptide with a compound suspected of having agonist or antagonist activity; and
 - (a) assaying for activity of said polypeptide.

<110> Human Genome Sciences, Inc.

<120> TGF-beta Receptor Polynucleotides, Polypeptides and Antibodies

<130> PT014PCT

<140> Unassigned
<141> 2000-08-10

<150> 60/148,682
<151> 1999-08-13

<150> 60/154,887
<151> 1999-09-20

<160> 31

<170> PatentIn Ver. 2.0

<210> 1
<211> 733
<212> DNA
<213> Homo sapiens

| | | |
|---|-----|--|
| <400> 1 | | |
| gggatccgga gccccaaatct tctgacaaaaa ctcacacatg cccaccgtgc ccagcacctg | 60 | |
| aattcgaggg tgaccgtca gtcttccctt tccccccaaa acccaaggac accctcatga | 120 | |
| tctcccgac tcctgaggtc acatgcgtgg tggggacgt aagccacgaa gaccctgagg | 180 | |
| tcaagttcaa ctggtagtgc gacggcgtgg aggtgcataa tgccaagaca aagccgcggg | 240 | |
| aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg caccaggact | 300 | |
| ggctgaatgg caaggagtagc aagtgcagg tctccaacaa agccctccca acccccattcg | 360 | |
| agaaaaaccat ctccaaagggc aaagggcagc cccgagaacc acaggtgtac accctgcccc | 420 | |
| catcccgaaa tgagctgacc aagaaccagg tcagcgtgac ctgcctggc aagggcttct | 480 | |
| atccaaagcg catcgccgtg gagtgggaga gcaatggca gccggagaac aactacaaga | 540 | |
| ccacgcctcc cgtgctggac tccgacggct ccttcttct ctacagcaag ctcaccgtgg | 600 | |
| acaagagcag gtggcagcag gggAACGTCT tctcatgctc cgtgatgcat gaggctctgc | 660 | |
| acaaccacta cacgcagaag agcctctccc tgctccggg taaatgagtg cgacggccgc | 720 | |
| gactcttagag gat | 733 | |

<210> 2
<211> 4087
<212> DNA
<213> Homo sapiens

| | | |
|---|------|--|
| <400> 2 | | |
| gcggggaggat gggccgcccgc taggctcgca ctccggacgc gcctcgcagt ggcgcagggtg | 60 | |
| gggtccccgc gcctcgacgc tccgggggg cggcgccggc ggaggtggcc gacaggctcc | 120 | |
| aggcctcgca gcctcggccc cccggccgcgc gcgttccgc acggcgccgc cgccgcgagc | 180 | |
| cacccggccc cccaagggtct ctgcggggcg ggagaacgga aaactcccaa cttcctgagt | 240 | |
| tctaaagttc ctgttgcattc agacaatggc tgagcaatca caaggaatgc aaggccacc | 300 | |
| tgttccctcg ttccaaccac agaaggcattt acgaccggat atgggctata atacattagc | 360 | |
| caactttcgca atagaaaaaa aaattggtcg cggacaattt agtggaaatgg atagagcagc | 420 | |
| ctgtctctt gatggagtagc ctagtagttt aaaaaaaaaatgg cagatattttt atttaatgg | 480 | |
| tgccaaagca cgtgctgtt gcatcaaaaga aatagatctt cttaaagcaac tcaaccatcc | 540 | |
| aatgtataata aatatattatg catcattcat tgaagataat gaactaaaca tagttttgg | 600 | |
| actagcagat gctggcggacc tatccagaat gatcaaggcat tttttaagaagc aaaagaggct | 660 | |
| aattcctgaa agaactgtttt ggaagtattt ttttgcattt tgcaatgcatt tggaacacat | 720 | |
| gcatttcgca agagtcatgc atagagataaa aaaaaccagct aatgtgttca ttacagccac | 780 | |
| tgggggtggta aaacttggag atcttgggtt tggccgggtt ttcaatgcattt aaaccacagc | 840 | |
| tgcacatctt ttagtggta cgccttata catgtcttca gagagaatata atggaaatgg | 900 | |
| atacaacttc aatctgaca tctgggtctt tggctgtcta statatgaga tggctgcatt | 960 | |
| acaaaagtct ttctatggtg aaaaaatgaa ttataactca ctgtgtaaag aatgatagaaca | 1020 | |
| gtgtgactac ccacctcttc cttcagatca ctattcagaa gaactccgac agttagttaa | 1080 | |

| | | | | | | |
|-------------|--------------|-------------|--------------|-------------|--------------|------|
| tatgtgcata | aacccagata | cagagaagcg | accagacgtc | acctatgtt | atgacgtagc | 1140 |
| aaagaggatg | catgcata | ctgcaagcg | ctaaacatgc | aagatcatga | agagtgtAAC | 1200 |
| caaagtatt | gaaagtattt | tgtcaagtc | atacctcccc | atttatgtct | ggtgttaaga | 1260 |
| ttaatatttc | agagctagt | tgcttgaat | ccttaaccag | ttttcatata | agcttcattt | 1320 |
| tgtaccatgc | acctaaaatca | cctcccttgca | acccccata | gactttggaa | taactgaatt | 1380 |
| gcatgttagg | agagaaaaatg | aaacatgtatg | gttttgaatg | gctaaagggt | tatagaattt | 1440 |
| cttacagttt | tctgtgtata | aatttgttt | agatagactg | tcagtgcata | atttgaaagg | 1500 |
| tgcagcttgg | cacacatcg | aatagactca | tacccgtgaa | aaagtatctg | aacatgtgac | 1560 |
| ttgtttctt | tttagtaatt | tatggacatt | gagatgaaca | caattgtgaa | cttttgtgaa | 1620 |
| gattttat | ttaaacgtt | gaagtactag | tttttagttct | tagcagagta | gtttcaaat | 1680 |
| atgattctt | tgataaaatgt | agacacaaaac | tatttgagaa | acatttagaa | ctcttagctt | 1740 |
| atacatcaa | aatgtacta | ttaaatgtga | agatttgggg | acaaaatgtg | agttagacac | 1800 |
| tgaagagttt | tttgggtt | ttaatattt | ttgatattct | cttgcattt | aaatgggtata | 1860 |
| aatgaatcca | ttttaaaatgt | ggttaaggat | ttgttttagt | ggtgtgat | aaatttttaa | 1920 |
| agttgcacat | tgcccaaggc | ttttttgtg | tttttttatt | ttttttgtt | cattttaaaa | 1980 |
| atattcttg | aataacctt | cagtactata | tttcaattt | tttataattt | taatgtcatt | 2040 |
| ttaactcata | attgtacact | ataatataag | cctaagttt | tattcataag | ttttattgaa | 2100 |
| gttctgatcg | gtcccccattca | gaaattttt | tatattattc | ttcaagttac | tttcttattt | 2160 |
| atattgtatg | tgcattttat | ccattaatgt | ttcatacttt | ctgagagtt | aataccctt | 2220 |
| taaaagat | ttggtatacc | aatactttt | ctggattgaa | aactttttt | aaacttttta | 2280 |
| aaattttggc | cactctgtat | gcatatgtt | ggcttggta | aagggaaaga | aaggatgtgt | 2340 |
| gttatactgt | acctgtat | tttgatatacg | tttacaattt | tttgatatacg | ttgttattct | 2400 |
| agaatatgt | taaaaaatgt | aaaactggcc | atgactacag | ccagaactgt | tatgagatta | 2460 |
| acatttctat | tgagaagctt | ttgagtaaag | tactgtattt | gttcatgaag | atgactgaga | 2520 |
| tggtaacact | tctgttagct | taaggaaatg | ggcagaattt | cgttaatgtct | gttgcgcaga | 2580 |
| tgtttttcc | ctgaatgtt | tcgttattgt | ggcgaccagt | ttctcacaga | attgtgaagc | 2640 |
| ctgaaggcca | agaggaagtc | actgttaaag | gactctgtgc | catcttacaa | ccttggatga | 2700 |
| attatccctgc | caacgtgaaa | acctcatgtt | caaagaaacac | ttcccttttag | ccgatgtaac | 2760 |
| tgctgggtt | gttttcata | tgttttttcc | tttacactcat | ttgaatgtt | tcaagcattt | 2820 |
| gtaaactaa | aaaatgtat | aaggggaaaa | agtcgtacc | tttgcattt | gaaatctaatt | 2880 |
| cagttatgt | tggttctga | agggtatattt | tattttggaa | taggtttttt | aaacctgttt | 2940 |
| tgtttgtttt | tcctgaggggc | tagatgtt | tttttctca | cactcttaat | gacttttaac | 3000 |
| atttatactg | agcatccata | gatatattcc | tagaagtatg | agaagaattt | ttcttatttga | 3060 |
| ccattaatgt | catgttcatt | ttaatgtat | ataatttggaa | ttgaatgtt | tctgtttggaa | 3120 |
| acagatactc | tctttttt | cttgcataat | ttaagaatac | atagatctaa | aatttattttag | 3180 |
| cttgaccctt | caaagtaact | ttaagttaaa | gattttttttt | tttcttctca | gttgcataat | 3240 |
| ctgcttagag | gaaatagctg | ggaagaaattt | aatgttgcgg | gaaatttattt | atttcttattt | 3300 |
| gtggaaactt | tttgcttgc | atattgtatc | ttttttttttt | taaatgttca | tatttttctt | 3360 |
| gaagaaacca | ctgtgtaaaa | atcaaatttt | aattttttttt | ggaataattt | caaagaacta | 3420 |
| tgaagatgt | tttgaaatgtt | aattttatata | gtcacatata | aatgttctt | tatgtgttt | 3480 |
| cataagtaaa | ttttatattt | attaagttaa | actttttttt | tgatgttgc | agcgtttttt | 3540 |
| tgaaaagctat | atcttttctt | aaccttattt | agacattttt | accgttacc | cagggttttt | 3600 |
| tatggat | ttttttttt | tatggat | tttgcattttt | gttgcattttt | gttatctctt | 3660 |
| tataataaaa | gtgtcaaca | atgtgcata | attttttttt | tagtgcatttt | ttacagccat | 3720 |
| ttcatgtat | ttttttttt | caacatgtt | tcttattttt | aacacccattt | ttgtatccat | 3780 |
| atacttcagg | tgttgcgtt | aacattttact | atgtatattt | ttttttttttt | aatgttactt | 3840 |
| acattaaatgt | tttattttttt | aaaatgtat | tattttttttt | ttaacccacaa | aatgcatact | 3900 |
| taccctgtgc | ctcatattt | aatgtactt | taatatggac | atttttttttt | aaatactttt | 3960 |
| attttgttat | gtttttttt | tacatataaa | aagattttttt | tttattttttt | tgaaaattttt | 4020 |
| ataatatcct | aatataaaaca | aaaatataaa | aataaaaaatgt | aatacagttt | aaaaaaaaaaaa | 4080 |
| | | | | | aaaaaaaaaaaa | 4087 |

<210> 3
<211> 2622
<212> DNA
<213> Homo sapiens

| | | | | | | |
|-------------|-------------|------------|-------------|-------------|-------------|-----|
| <400> 3 | 60 | | | | | |
| gcggaaatgtt | caactccaa | ctgcggcgga | aacgtggag | ccgcgcgggc | cgctgtcg | 60 |
| ccaaaccccg | ccgcctcg | cgcgccgg | gcctccgcgc | ccccggctgc | tgctcacg | 120 |
| ccgccccggg | gcacatgtt | gtggaaatgt | aatactttgt | cattatgtt | tgtcgctct | 180 |
| cggtgcctcc | tttgcgtt | ttaaatttgc | tgacttgc | ttttttttttt | actgcgg | 240 |
| aggaatgttt | gggatgtttt | atcgagccaa | atgtatatac | caggacaagg | agttggctgt | 300 |
| aaagaagctc | ctcaaaaatag | agaaagaggc | agaaataactc | agtgtccct | gtcacagaaaa | 360 |
| catcatccag | tttatgttgc | accccaac | tatggcattt | tcacagaata | | 420 |

| | | | | | | |
|-------------|-------------|-------------|-------------|-------------|--------------|------|
| tgttctctg | ggatcactct | atgattacat | taacagtaac | agaagtgagg | agatggatat | 480 |
| ggatcacatt | atgacactggg | ccactgtat | agccaaagga | atgcattatt | tacatatgga | 540 |
| ggctcctgtc | aaggtgattc | acagagacct | caagtcaaga | aacgttgtt | tagctgtga | 600 |
| tggagtattg | aagatctgtg | actttggtc | ctctcggtc | cataaccata | caacacacat | 660 |
| gtccttgggt | ggaactttcc | catggatgc | tccagaagt | atccagagtc | tccctgtgtc | 720 |
| agaaaactgt | gacacatatt | cctatgggt | ggttctctgg | gagatgtcaa | caaggaggt | 780 |
| ccccctttaaa | ggttggaaag | gattacaat | agcttgggt | tgatgtggaa | aaaacgagag | 840 |
| attaaccatt | ccaagcagtt | gccccagaag | ttttgctgaa | ctgttacatc | agtgtggga | 900 |
| agctgtatgc | aagaaacggc | catcattca | gcaaatcatt | tcaatcctgg | agtccatgtc | 960 |
| aatgacacg | agccttcctg | acaagtgtaa | ctcattccta | cacaacaagg | cggagtggag | 1020 |
| gtgcgaaatt | gaggcaactc | ttgagaggt | aaagaaaacta | gagcgtgatc | tcagctttaa | 1080 |
| ggagcaggag | cttaaagaac | gagaaagacg | tttaaagatg | tgggagcaaa | agctgacaga | 1140 |
| gcagtccaa | accccgctgc | tgccttcctt | tgagattgt | gcatggacgg | aagacgtat | 1200 |
| gtattttgg | gttcagcagc | tcgtcagaaa | aggtgactt | tcagcagaga | tgagtgtata | 1260 |
| tgcaagctt | ttttaaagaaa | acaacattac | agggaaagcg | ctgctgctgc | tgaggaga | 1320 |
| agacctgaaa | gacatggca | ttgtctccaa | ggggcatatc | attcacttca | agtcaaggccat | 1380 |
| tgagaattt | acccatgatt | acataaattt | gtttcacttc | ccaccactaa | ttaaggactc | 1440 |
| aggaggtgaa | cctgaagaaa | atgaggaaaa | aatagtgaac | ctggaactgg | tttttggtt | 1500 |
| tcacttgaaa | ccaggaactg | gcccacagga | ttgttaagtgg | aaaatgtata | tggagatgga | 1560 |
| tggggatgaa | attgcaataa | cctacataaa | agatgtgaca | ttcaacacta | acctacactg | 1620 |
| tgccggagtt | ttaaagatg | caaagccacc | attttaatg | gagaagtgg | ttttaggaat | 1680 |
| agaaaaaaat | cagactgtgg | atgtcaact | catatatgag | agtgtgtt | gaactccaaa | 1740 |
| aagactaaa | catgtccatt | tgattcaatg | gagtagaaca | aaacctcaagg | atgaagtgaa | 1800 |
| agcagttccaa | cttgccattc | agacattatt | caccaattca | gatggcaacc | ctggaaagcag | 1860 |
| gtccgactca | agtgtctgatt | gccagttgtt | agataactctg | aggatgcggc | agattgcatc | 1920 |
| caacacttct | ttacagcggt | cccagagcaa | tccttattctg | gggtcacccgt | tcttctcaca | 1980 |
| ctttatggc | caggattctt | acgctgtgc | tgtgagacgg | ccccaggtgc | ccattaagta | 2040 |
| tcaacagatt | acacctgtga | accgttcag | aagtcgtct | cctactcaat | atggactgac | 2100 |
| caaaaacttc | tcttccctac | atctcaatc | tagggacagt | ggctttccca | gtggcaatac | 2160 |
| tgacacctt | tcagagaggg | gtcgataatc | agacagaagc | aggaacaaat | atggacgtgg | 2220 |
| tagtatatca | ctcaattctt | ctccttagagg | aagataatg | ggaaagagtc | agcattccac | 2280 |
| tccttcaaga | ggaagatacc | ctggaaagtt | ctacagggtt | tctcagtcag | cactcaatcc | 2340 |
| tcaccagtcg | cctgacttca | agagaagccc | cagggacctc | caccaaccca | acaccatacc | 2400 |
| aggatgcct | ttgcaccctg | agactgactc | aagagccat | gaagaggaca | gcaaagttag | 2460 |
| cgaagggggg | tggacaaaaag | tggataatcg | gaaaaagccc | cacaggccat | ctcccgccaa | 2520 |
| aaccaataaa | gagagagcca | gaggggacca | ccgtggatgg | agaaaacttt | gatgaattga | 2580 |
| actacatagc | ttttctaagc | aggttaaaaa | aaaaaaaaaa | aa | | 2622 |

<210> 4
<211> 3283
<212> DNA
<213> Homo sapiens

| | | | | | | |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| <400> 4 | | | | | | |
| catcaatcga | gcccagggtcc | cagccaccac | cactcacagc | gctcggcggt | caggaagagg | 60 |
| agcagcagcg | gaggcggctg | cttcagcggc | gggcgggcgc | cagaaaggcc | ccgatcgaaa | 120 |
| acgcctgggg | ggccggccgaa | ctaccccccgg | aggaggagc | cagtccgaac | ccaaggcgcc | 180 |
| accggccgag | aagcgagcg | aggcagatt | cgccctccatg | gcccactcg | cgggtggctgt | 240 |
| cgaatgcct | gggatcgaga | ataacatagc | tgatccagaa | gaactgttca | aaaaattaga | 300 |
| gcgcattggg | aaaggctcat | ttggggaaat | tttccaaagga | attgataacc | gtacccagca | 360 |
| agtcgttgc | attaaaatca | tagaccttga | ggaagccaa | gatgaaatag | aagacattca | 420 |
| gcaagaaaata | actgtcttga | gtcaatgtga | cagctcatat | gtaacaaaat | actatgggtc | 480 |
| atatttaaag | gggtctaaat | tatggataat | aatggaatac | ctgggcccgt | tttcagcact | 540 |
| ggatcttctt | cgagctggtc | catttgcata | gttccagat | gctccatgc | taaaggaaat | 600 |
| ttttaaaggt | ctggactatc | tgcattcaga | aaagaaaatt | cacccgagaca | taaaagctgc | 660 |
| caatgtcttgc | ctctcagaac | aaggagatgt | taaaatgt | gatttggag | ttgtgttca | 720 |
| gctgacagat | acacagatta | aaagaaaatac | ctttgtggga | actccatttt | ggatggctcc | 780 |
| tgaagttatt | caacagtca | cttatgactc | aaaagctgac | atttggtcat | tgggaaattac | 840 |
| tgctattgaa | ctagccaagg | gagagccacc | taactccat | atgcattccaa | tgagagttct | 900 |
| gtttcttatt | cccaaaaaca | atccctcaac | tcttggatgg | gactttacta | agtcttttaa | 960 |
| ggagttttt | gtatgttgc | tgaacaaaga | tccatcattt | cgtccctacag | aaaaagaact | 1020 |
| tctgaacac | aaatttattt | taaaaaattt | aaagaagact | tcttattctga | ctgaactgat | 1080 |
| agatcggttt | aagagatgga | aggcagaagg | acacatgtat | gatgaatctg | attccgagggg | 1140 |
| ctctgattcg | gaatctacca | cgaggaaaa | caatactcat | cctgaatgga | gcttaccac | 1200 |
| cgtacgaaag | aagcctgatc | caaagaaaat | acagaatggg | gcagagcaag | atcttgcga | 1260 |

| | | | | | | |
|--------------|-------------|------------|-------------|-------------|-------------|------|
| aaccctgagt | tgtttgtcta | tgataatcac | acctgcattt | gctgaactta | aacagcagga | 1320 |
| cgagaataac | gctagcagga | atcaggcgat | tgaagaactc | gagaaaagta | ttgctgtggc | 1380 |
| tgaagccgcc | tgtccggca | tcacagataa | aatggtaag | aaactaatg | aaaaatttca | 1440 |
| aaagtgttca | gcagacgaat | ccccctaaga | aacttattat | tggcttctgt | ttcatatgga | 1500 |
| cccagagagc | cccaccaaac | ctacgtcaag | attaacaatg | cttaaccat | gagctccatg | 1560 |
| tgccttttgg | atctttgcaa | cactgaagat | ttgaaagaag | cttataact | atttgtat | 1620 |
| ggcgtttatac | attttatatt | ttgaaagat | tatTTTgaa | ggaataactt | ttaatactat | 1680 |
| agtttccac | gtattctagt | aaatgtttag | acaccgttt | gcttttaagt | atccctattt | 1740 |
| cttaagttac | gaggatgaat | accttcaca | ttttgatctt | tagttgactc | tacagtcatg | 1800 |
| aaacatatacg | gtctttcaaa | gtcattotca | atattcagct | tttggaaatt | atcaagcttc | 1860 |
| aaaaagctt | ttttaaaaaa | aaaaaacatg | catattctaa | aaatgactat | tggtggggag | 1920 |
| gtgtaaataa | gtcatacatt | cttaaaacag | aaaatttaag | taaagtctt | taaatgaaac | 1980 |
| ctgtaaaaat | attgactt | ctaccaagg | ggatgat | tccaggcagc | tcaatgatta | 2040 |
| tcacatttga | gaccctgtgt | tttggagat | tacaggcaat | gtacagcaac | agaggtaacct | 2100 |
| cttgggttat | atgatattaca | ttctcttta | ggtagaaagag | gcaattttac | ccttatttca | 2160 |
| catggtaga | aattttaaagc | aagatcattt | acccaaggat | agggttttg | taatgttga | 2220 |
| ggagtttagt | tggcttcatg | ttttacatct | tcaactaaaa | tcccataacta | tctgcttgg | 2280 |
| tttggagagc | caaaaaataa | agctgattgt | catgtgatta | aatatctgtat | caacaggtat | 2340 |
| gaatataact | taaatcagca | tatTTTgca | atggtaataa | atgttcttat | aaactattt | 2400 |
| tatatttttg | tttccatcaa | tttacactaa | taagcatcag | tttgggtttt | ttaaaaggat | 2460 |
| atttaaatgt | gtatTTtca | tttccatata | aaataaccat | agtaggat | gatttctgtc | 2520 |
| cacacaaagg | ttaaatttga | tttccatata | tttccatata | tatattttag | gtactattat | 2580 |
| gtgggtat | cctttttctt | tttccatata | tttccatata | atgcctgcct | aagtctgaa | 2640 |
| ctggggctgt | atttcagtag | tttccatata | tttccatata | atgttgcata | gctaatgttt | 2700 |
| aattgtttgg | atctgcacag | tttccatata | tttccatata | cattttaaa | aatctgagta | 2760 |
| attgtcaat | attttttttt | tttccatata | tttccatata | tttccatata | tttccatata | 2820 |
| ggccattaca | tcctctttt | tttccatata | tttccatata | tttccatata | tttccatata | 2880 |
| ttgtatgcca | agaaaaattct | tttccatata | tttccatata | tttccatata | tttccatata | 2940 |
| ttgtatTTTg | aagtaacgg | tttccatata | tttccatata | tttccatata | tttccatata | 3000 |
| tacagttat | aatcatctgt | tttccatata | tttccatata | tttccatata | tttccatata | 3060 |
| aatgaatatt | tttccatata | tttccatata | tttccatata | tttccatata | tttccatata | 3120 |
| caacacaatt | gtatccc | tttccatata | tttccatata | tttccatata | tttccatata | 3180 |
| taatctgttt | cttaggtgaag | tttccatata | tttccatata | tttccatata | tttccatata | 3240 |
| aatttccatata | tttccatata | tttccatata | tttccatata | tttccatata | tttccatata | 3283 |

<210> 5
<211> 3200
<212> DNA
<213> Homo sapiens

| | | | | | | |
|-------------|--------------|--------------|-------------|-------------|-------------|------|
| <400> 5 | | | | | | |
| gaagaagcag | gccccgtgaagc | ggcaccacca | caagcacaac | ctggggcacc | gctacgagtt | 60 |
| cctggagacc | ctggggcaaaag | gcacctacgg | gaaggtgaag | aaggcgccgg | agagctcg | 120 |
| gcccctgg | gccatcaagt | caatccggaa | ggacaaaatc | aaagatgagc | aagatctgtat | 180 |
| gcacatacgg | agggagattt | agatcatgtc | atcactcaac | caccctcaca | tcatttgcatt | 240 |
| ccatgaagt | tttggaaaca | gcagcaagat | cgtgatcg | atggatgtat | ccagccgggg | 300 |
| cgacccat | tttggatgtat | gactacatca | gcgagcggca | gcagctcagt | gagcgcgaag | 360 |
| cttccggcag | atctgtctgt | ccgtgcacta | tttggatgtat | tttggatgtat | tttggatgtat | 420 |
| tctcaagct | gagaacatcc | tcttggatgtat | caatggaaat | atcaagat | ctgacttcgg | 480 |
| cctctccaa | cttaccatc | aaggcaagtt | cctgcagaca | tttggatgtat | tttggatgtat | 540 |
| tgcctcggca | gagattgtca | atgggaagcc | ctacacaggc | ccagggatgg | acagctgg | 600 |
| cctgggtgtt | ctccctctaca | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 660 |
| taatgttca | gtgaaacaga | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 720 |
| tgcctgtggc | ctgatccgg | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 780 |
| ggatgtggcc | agtactgtt | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 840 |
| ggctccgcatt | gagggtgggc | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 900 |
| gctccggcgt | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 960 |
| gcagcatgca | cctgggtgggg | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1020 |
| gaagtcccgc | aaggagaat | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1080 |
| tgcctcgc | cctggcaaga | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1140 |
| gtcagctct | gcagaagggg | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1200 |
| cccaggcag | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1260 |
| tgagtctggc | tactactt | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1320 |
| cgacgtgttt | gtgagtgccc | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1380 |
| cctccatcgc | aaaggcatcc | tttggatgtat | tttggatgtat | tttggatgtat | tttggatgtat | 1440 |

| | |
|---|------|
| cgccggccccc accaccttcg gctccctgga tgaactcgcc ccacctcgcc ccctggcccg | 1500 |
| ggccagccga ccctcagggg ctgtgagcga ggacagcatc ctgtcctctg agtccttga | 1560 |
| ccagctggac ttgcctgaac ggctccccaga gcccccaactg cggggctgtg tgtctgtgga | 1620 |
| caacctcacg gggcttgagg agccccccctc agagggccct ggaagctgcc tgaggcgtg | 1680 |
| gccgcaggat cctttggggg acagctgtt tccctgaca gactgccagg aggtgacagc | 1740 |
| gacctaccga caggcactga gggctctc aaagctcacc tgagtggagt aggcatgcc | 1800 |
| ccagcccggt caggctctc catgcagctg gttgcacccc gaggggagat gccttctccc | 1860 |
| ccacccctca ggapctcat cccagctcag aaggctgaga gggtttgcag tggagccctg | 1920 |
| agcagggtcg gatatggaa gtaggcaaat gaaatgcgc aagggttcag tgtctgtctt | 1980 |
| cagccctgct gaacgaagag gatactaaag agagggaaac gggaatgcc ggcacagagt | 2040 |
| ccacattgcc tggttcttgt gtacatgggg gggccacaga gacctggaaa gagaactctc | 2100 |
| ccagggccca tctctgtcat cccatgaata ctctgtacac atgggtgcctt ctaaggacag | 2160 |
| ctcctccctt actcattccc tgcccaagtg gggccagacc tctttacaca cacattcccg | 2220 |
| tccatcccaa ccaccagaac tggatgggtg cacccttaat gtgcattgagg catcctggga | 2280 |
| atggctggaa gtaacgcttc gttatttta tttttatttt tatttttta tttttttttt | 2340 |
| tgagacggag tttcgctttt ggtgcccagg cttagagtca atggcgcat ctcagctcac | 2400 |
| ctcaacctcc gcctccccc ttcaagcgat tctctgcct cagcctccct agtagctggg | 2460 |
| attacaggcg cccgcccacca tgcccggcta attttgtatt ttttagtagag acagggtttc | 2520 |
| tccatgttgg tcaggctgtt ctcaaaactcc cgacccctcagg tgatccaccc acctcggccct | 2580 |
| cccaaagtgc tgggattaca ggcgtgagcc accgcggccc acctaaccct tccttattta | 2640 |
| gccttaggat aagagaacac aatctctgtt tcttcattgg ttcttcttcc ttttccatcc | 2700 |
| tccaaacctg gcctgagcct cctgaagttt ctgtgtgaa tctgaaaagac ttgaaaagcc | 2760 |
| tccgcctgct gtgtggactt catctcaagg ggcccagct cctctggact ccacccctgga | 2820 |
| cctcagtgac tcagaacttc tgccctctaag ctgctctaaa gtccagacta tggatgtgtt | 2880 |
| ctctaggcct tcaggactct aagaatgtcca tattttattt tatgttctt gctttgtgtt | 2940 |
| tttagaaaaag tgaatcttgc tggtttcaat aatgtgaatg ctatgttctg ggaaaatcca | 3000 |
| ctatgacatc taagttttgt gtacagagag atattttgc aactatttcc acctccctccc | 3060 |
| acaacccccc acactccact ccacactctt gagtctttt acctaattgtt ctctacctaa | 3120 |
| tggacccccc tggccaaaaaa gtaccattaa aaccagaaag gtgattggaa aaaaaaaaaa | 3180 |
| aaaaaaaaaa aaaaaaaaaa | 3200 |

<210> 6
<211> 2333
<212> DNA
<213> Homo sapiens

| | |
|---|------|
| <400> 6 | |
| tgcggctccg gcccgtcg cgtgggggtc cgggtgttcg cggtcggccg ccaccaggtc | 60 |
| accctgaaag agtcgctggc cgaagggtgga ttctccacag ttttcctgtg gctactcac | 120 |
| ggtggaatcc gatgtgcatt gaagcgaatg tatgtcaata acatgccaga cctcaatgtt | 180 |
| tgtaaaaggg aaatttacaaat tatgaaagag ctatctggc aaaaaatataat tggggctat | 240 |
| ttggactgtg ctgttaattc aatttagtgc aatgtatggg aagtccttat tctaatggaa | 300 |
| tattgtcgag ctggacagggt agtgaatcaa atgaataaga agctacagac gggttttaca | 360 |
| gaaccagaag tggtagatcattctgtat acctgtgaag ctgttgcag gttgcattcag | 420 |
| tgttaagactc caataattca cccggatctg aaggtagaaa atattttgtt gaatgatggt | 480 |
| gggaactatg tactttgtg ctttggcagt gccactaata aatttcttaa tcctcaaaaaa | 540 |
| gatggagttt atgtatgtt agaagaaatt aaaaaggact gggatgttca ctctataaac | 600 |
| tttggttttt cacttttctt tttgggtgaga gtcaagggttgc tatctgtat ggcaacttca | 660 |
| ccatcccaaga caattctcgta tactcccgtt acatacattt cttaaagggttca ttcatgctt | 720 |
| aaccagatcc ggaacataga cctgtatata ttcaagtgtc atatttttgc tttaaattttt | 780 |
| ccaaaaagga ttgtccagtc tccaaacatca ataatttttc tatttcctca gctttcctt | 840 |
| aaccgatgac tgcgtatgtt gcaagctgtt gggaaaagcca aataaaagcc agaataacag | 900 |
| ataccattgg accaacacaaa acctcaattt caccacatca aagaccaaaag gccaactctt | 960 |
| ctactacttc cactcccaatgttgcgttacca gcaacactt acgcaacactt tttaaggatc | 1020 |
| ttgtctctgg tgaatttgcgtt aaccatagac caaaaggggc actaagactt ggaatggcc | 1080 |
| ctgaaaatttt atgggttcgtt ggaccccttc acagccgc acagcagcat agactactcc | 1140 |
| agcaactaca gcagggtatc tggagattac agcaacttca ttacatgcgtt cgtcatcttc | 1200 |
| accagcagca gcagcagcagc cagcaacacgc aacagcagca gcagcaacag caacagcagc | 1260 |
| agcagcagca gcagcagcagc caccacccacc accacccaca ccaccaactt caagatgctt | 1320 |
| atatgcagca gtatcaacat gcaacacacgc agcaacacat gcttcaacaa caattttaa | 1380 |
| tgcattccgtt atatcaacca caaccccttc catcacatca ttctacaatg atgcgcgtt | 1440 |
| atcagcaggc ttctttcaat cagcagatgc tagtcaaca tcagccgttcaatcacaacagg | 1500 |
| catcacatca atatcttacc tccctcaag agtttcacc agccttagt tcctacactt | 1560 |
| catcacatcc agctcaggat ggaaccatca tggactcttc ctatgtgcc aataggtcag | 1620 |
| ttgctgataa agaggccatt gcaaaatttca caaatcagaatc aatccacatcg | 1680 |

| | | | | | | |
|-------------|-------------|------------|-------------|-------------|-------------|------|
| atatgtcagg | gtggaatcct | tttggagagg | ataatttctc | taagttaaca | gaagaggaac | 1740 |
| tattggacag | agaatttgac | cttctaagat | caagtaaggg | acacttgaag | gcttattttg | 1800 |
| cttcacagta | aaataaacgc | tctattatta | ttcagcaagg | ccaaaagactt | ttgagaatgt | 1860 |
| gtatggaaaa | ttctttttgt | gcatttgagg | gcaaaaattca | ggccatcttc | ttatacatat | 1920 |
| actatcaaat | tatgttgtgt | gcatttagaa | tcagttgtt | gatagtagct | attaaaccca | 1980 |
| atattgtga | tagtatgtct | atatcctaaa | acttaaatat | tgcatatcta | tgaatgttaa | 2040 |
| attcagaata | tctctaaaac | atggaaaat | gatgtttca | aaaaaaggga | gacattttat | 2100 |
| tattttgccct | tactaatgtat | tttgagctc | tgttttctg | cacactcaat | agaaaaatatt | 2160 |
| gtgggtctga | gatgcccttt | gaaaatgcct | gaaagaaaac | atgggctact | acattatgtt | 2220 |
| aatgtttgt | aatgtccctt | taatgagtt | tgacataaaa | gggctgttt | gttgcctgaa | 2280 |
| tatggcaaaa | attaaataaa | ttcttttca | ctttaaaaaa | aaaaaaaaaa | aaa | 2333 |

<210> 7
<211> 1490
<212> DNA
<213> Homo sapiens

| | | | | | | |
|-------------|-------------|-------------|-------------|-------------|--------------|------|
| <400> 7 | | | | | | |
| tggacctgct | actggggcggt | gacctgcgt | accacctgc | gcagaacgtg | cagttctccg | 60 |
| aggacacgg | gaggctgtac | atctgcgaga | tggactggc | tctggactac | ctgcgcggcc | 120 |
| agcacatcat | ccacagagat | gtcaagcctg | acaacattct | cctggatgag | agaggacatg | 180 |
| cacacctgac | cgacttcaac | attgccacca | tcatcaagga | cggggagcgg | gcgacggcat | 240 |
| tagcaggcac | caagccgtac | atggctccgg | agatcttcca | ctctttgtc | aacggcggga | 300 |
| ccggctactc | cttcgaggtg | gactgggtt | cgggtgggt | gatggcctat | gagctgctgc | 360 |
| gaggatggag | gcccattagac | atccacttca | gcaacgcgt | ggagttccctg | gtgcagctgt | 420 |
| tcagcacccgt | gagcgtccac | tatgtccccca | cgtgttccaa | ggagatgggt | gccttgcgtc | 480 |
| ggaagctctt | cactgtgaac | cccgagcacc | ggcttccag | cctccaggac | gtgcaggcag | 540 |
| ccccggcgt | ggccggcgt | ctgtgggacc | acctgagcga | aaagagggtg | gagccgggct | 600 |
| tcgtccccaa | caaaggccgt | ctgcactgct | accccaccc | tgagctggag | gagatgatcc | 660 |
| tggagttccag | gcccattgcac | aagaagaaga | agcgcctggc | caagaacaag | tccgggaca | 720 |
| acagcaggga | cagctccca | tccgagaatg | actatcttca | agactgcctc | gatgcccattcc | 780 |
| agcaagactt | cgttattttt | aacagagaaa | agctgaagag | gagccaggac | ctcccgagg | 840 |
| acgcctctcc | gcccattgt | ccagggatgc | tgccggaccc | ttggaggacg | aggcggaaacg | 900 |
| ctccgcctg | cccatgtgc | gccccatcc | ccccctggcc | gggagcggct | aggccggat | 960 |
| gccccgtgtc | ctcaccctt | gagctgttt | ggagactcgg | ctgcccagg | gagggccatg | 1020 |
| ggcccgaggcc | tggcattcac | gttcccaccc | agctggctg | gccccgtgtc | cagtccccg | 1080 |
| gacacatttc | acacctcagg | ctcggttgg | tgccggggac | aagaggctgt | gggtgcagg | 1140 |
| gacacctgtg | gagggcattt | cccggtggcc | cccgagaccc | gcctagatgg | aggaagcgct | 1200 |
| gctgggcggc | ctcttaccgc | tcacggggag | ctggggccat | ggatggaca | ggagtttttgc | 1260 |
| tccctgtctc | gccccggagc | tgtgcaccc | cctctgtcaca | aggtgaccct | tgccggcggc | 1320 |
| gcccgggtg | ccccaggctc | ggctcagttc | ttggaggct | agggcatgg | ttggggtagt | 1380 |
| gggtggggag | gtgaatgtt | tctagagatt | caaactgctc | cagcaatttc | tgtatagtt | 1440 |
| tcacctctga | gaattacaat | gtgagaaccc | ctaaaaaaa | aaaaaaaaaa | | 1490 |

<210> 8
<211> 1501
<212> DNA
<213> Homo sapiens

| | | | | | | |
|-------------|-------------|-------------|-------------|-------------|------------|-----|
| <400> 8 | | | | | | |
| acgaggtaact | ttgtgatgga | ctacatccct | ggggggaca | tgtatggcct | gtgtatccgg | 60 |
| atggagggtct | ccctctgagca | cctggccgg | ttctacatcg | cagagctgac | tttggccatt | 120 |
| gagagtgtcc | acaagatggg | cttcatccac | cgagacatca | acgcctgataa | cattttgata | 180 |
| gatctggatg | gtcacattaa | actcacatg | ttccgcctct | gcactgggtt | cagggtgact | 240 |
| cacaatttca | aatattacca | gaaaggggac | catgtcagac | aggacacatg | ggagccacg | 300 |
| gacctctggg | atgatgtgtc | taactgtccg | tgtggggaca | ggctgaagac | cctagagcag | 360 |
| agggcgccga | agcagcacca | gagggtgcctg | gcacattcac | tggggggac | tccaaactac | 420 |
| atcgcaccccg | aggtgctctt | ccgcaaaagg | tacactcaac | tctgtgactg | gtggagtgtt | 480 |
| ggagtgattc | tcttcgagat | gctgggtggg | cagccgcct | ttttggcacc | tactccca | 540 |
| gaaaccggc | tgaaggtgt | caactgggg | aacacgtcc | acattccacg | ccaggtgaag | 600 |
| ctgagccctg | aggccaggga | cctcatcacc | aagctgtgt | gtcccgacaa | ccaccgcct | 660 |
| gggcggaaatg | ggggccatgt | cctgaaggcc | cacccttct | tcagcccat | tgacttctcc | 720 |
| agtgacatcc | ggaagcagcc | agccccctac | gttccacca | tcagccaccc | catggacacc | 780 |
| tcgaatttcg | accccgtaga | tgaagaaagc | ccttggaaacg | atgccagcga | aggtacacc | 840 |

| | | | |
|------------------------|-------------------------|-----------------------|------|
| aaggccctggg acacactcac | ctcgccccat aacaagcatc | ctgagcacgc attttacgaa | 900 |
| ttcacccccc | gaagggttctt tgatgacaat | ggctaccctt ttgcgtcccc | 960 |
| ggagcagaag | cttcacaggc tgagagctca | gatttagaaa gctctgatct | 1020 |
| actgaaggct | gccagccctgt gtacgtgtag | atggggcca ggcaccccca | 1080 |
| cctcccaaggt | cagggtccccg gagccgggtgc | cctcacaggc caataggaa | 1140 |
| gtttttttt | aaatttagtcc gtcgattact | tcacttgaaa ttctgctctt | 1200 |
| acccaaacag | gacacttttggaaacagac | tccatcgcc tttcaatagg | 1260 |
| ccttcactgc | attaaaacaa tatTTTgaa | atttagtac agtttagaaa | 1320 |
| tttggttata | tccattttt cttactaaat | tataggatt aactttgaca | 1380 |
| ctgttatttt | ctacatttgt attttatcca | tagacttat tcacatttag | 1440 |
| a | aaaaactgaa gaacattgtat | gagaatctc tgtcaataa | 1500 |
| | | tgtaaaaaaaaaaaaa | 1501 |

<210> 9
<211> 2331
<212> DNA
<213> Homo sapiens

| | | | |
|-----------------------------|-------------------------|--------------------------|------|
| <400> 9 | | | |
| cggacggcgtg ggctgccccggg | ctcgccgtga gtcgctgcgg | ggctgacggg gtggcagtgc | 60 |
| ggcgggttac ggcctggta | gaccataatg acttcagcaa | ataaaagcaat cgaattacaa | 120 |
| ctacaagtga aacaaaatgc | agaagaatta caagactta | tgccggattt agaaaactgg | 180 |
| aaaaaaagaca taaaacaaaaaa | ggatatggaa ctaagaagac | agaatggtgt tcctgaagag | 240 |
| aatttacccctt ctattcgaaa | tgggaattt aggaaaaaaa | agaaaaggccaa agctaaagag | 300 |
| tcttccaaaaaa aaaccagaga | ggaaaacaca aaaaacagga | taaaatcta tgattatgag | 360 |
| gcatggccaa aacttgcgt | ggaccgtatc cttgtatgc | ttgacaaaaga cgatagtacc | 420 |
| catgatctc tgcgtcaaga | atcagatcg gaagaagat | ggattcatgt agattcacaa | 480 |
| aaggcttgc tttaaaaaaaa | aaaggccaat aaatactca | aacaaggaaa atatgtgaa | 540 |
| gcaatttgcgt gctacacaaa | aggcatggat gcccattccat | ataatccctg gttgccaacg | 600 |
| aacagagcgt cagcatat | tagactgaaa aaatttgcgt | ttgctgagtc tgattgtat | 660 |
| tttagcgttg ctttgcata | aagtataca aaggcttatt | ccagacgagg tgctgctcga | 720 |
| tttgcttgc aaaaatttgc | agaggccaaa aaagattatg | aaagagtattt agaaactagaa | 780 |
| ccaaactaact ttgaagcaac | aatgaactc agggaaatca | gtcaggctt agcatccaaa | 840 |
| gaaaactcat atccaaagga | agctgacat gtgattaatg | caacaaaggg agagcggaaag | 900 |
| caaatttgcata cacaacagaa | taagcagcag gccatttcag | agaaagatcg gggaaatgga | 960 |
| tttttccaaag agggaaaata | tgaaagagca attgaatgc | atactcgagg gatagcagca | 1020 |
| gatgggtcta atgcccattt | tccagctaac agagctatgg | cctatctgaa gattcagaaa | 1080 |
| tatgaagaag ctgaaaaaaa | ctgcacacaa gccattttat | tagatggctc atattctaaa | 1140 |
| gcttttgcacaa gaagggaaac | tgcaagaaca ttttggaa | agctaaatga ggcacaaaacaa | 1200 |
| gatttttttttgcata ctgttttact | tctggaaacct ggaataaagc | aaggcgttac tgaactctcc | 1260 |
| aaaattaaaaaa aggaattat | tgagaaagga cactggatg | atgtctttt tgattccaca | 1320 |
| caaagacaaa atgtggtaaa | accattgtat aatccaccgc | atccctggatc aactaaacca | 1380 |
| ctcaagaagg ttattattgt | agaaaactggt aatttgatac | agactattga tgcgtccagat | 1440 |
| agcactactg ctgctgctcc | agagaataat cctattatc | tagcaaatgt aatagcagcc | 1500 |
| acaggccacca caagtaagaa | gaattcaagc caagatgacc | tttttccac aagtgtatact | 1560 |
| ccaaagacaa aagtattgaa | aatagaagaa gtcagtata | cttcattccct gcaacacctaa | 1620 |
| gccagttgtc acaggatgt | atgtcagtct tacatcgaga | aatggccat agagatagaa | 1680 |
| aaaaaaacctg cttagttgc | cacaactt cttccatccaa | ttccctgcata ctcgttccag | 1740 |
| ctcgaatctg atttcagaca | attggaaaatg tccctcagata | ttgtgtatca gtattttaaag | 1800 |
| caaatttgcata catctttgt | tccttaagttt tttcagaaaa | atctggatcc agatgtattc | 1860 |
| aaccagatcg taaaatttct | gcatgacttt tacattgaga | aagaaaagcc attactcatc | 1920 |
| tttggaaatct tacaagact | ttctgaacta aaaaggttt | atatggcaat gatgtttatg | 1980 |
| tcagaaacag agaaaaaaat | tgcacgtgca ttatatac | acatagacaa gtcaggattg | 2040 |
| aaggatagtt ctgtcgaaga | actcaagaaa agatacggtg | tttgatccc atttttgcgt | 2100 |
| aaataattgt ttggacttt | catatgtaaa ttggatccc | ttggatggatc ttgctttta | 2160 |
| agaaaaatgaa attatatac | aggaaaggac tatcttgc | cataagttaa ttaactataa | 2220 |
| ggtgaattgt gatTTTacta | gtgagaattg tattcaatgt | aactctgtt ttctgaaaat | 2280 |
| aaaaatataa acaatgarat | acaaaaaaaaaaaaaa | aaaaaaaat aaaaaaaaaaaaaa | 2331 |

<210> 10
<211> 3515
<212> DNA
<213> Homo sapiens

<400> 10

| | | | | | | |
|-------------|--------------|-------------|--------------|-------------|-------------|------|
| cagaactact | gctaggagag | gaacgttaca | caccagccat | agatgttgg | agctgtggat | 60 |
| gtattcttgg | ggaactattc | acaaagaagc | ctatTTTca | agccaatctg | gaactggctc | 120 |
| agctagaact | gatcagccga | ctttgtgtta | gcccttgc | agctgtgtgg | cctgtatgtt | 180 |
| tcaaactgcc | ctacttcaac | accatgaac | cgaagaagca | atatcgaaagg | cgtctacgag | 240 |
| aagaattctc | tttcatttct | tctgcagcac | ttgttattt | ggaccacatg | ctgacactag | 300 |
| atccttagta | gcccgtgcaca | gctgaacaga | ccctacagag | cgacttccctt | aaagatgtcg | 360 |
| aactcagcaa | aatggctctt | ccagaccc | cccactggca | ggattggcat | gagttgtgga | 420 |
| gtaagaaacg | gcgacgtcg | cgacaaagt | gtgttgtagt | cgaagagcca | cctccatcca | 480 |
| aaacttctcg | aaaagaaaact | acctcaggg | caagtactga | gcctgtgaag | aacagcagcc | 540 |
| cagcaccacc | tcagcctgtc | cctggcaagg | tggagtctgg | ggctggggat | gcaataggcc | 600 |
| ttgctgacat | cacacaacag | ctgaatcaa | gtgaatttgc | agtgttata | aacctgtgc | 660 |
| agagccaaac | cgacccgtgc | atccctcaaa | tggcacagct | gtttaacatc | cactccaacc | 720 |
| cagagatgc | gcagcagctg | gaagccctga | accaatccat | cagtgcctg | acggaaagcta | 780 |
| cttcccagca | cgaggactca | gagaccatgg | ccccagagga | gtctttgaag | gaagcacccct | 840 |
| ctgccccagt | gatcctgcct | tcagcagaac | agacgaccct | tgaagcttca | agcacaccag | 900 |
| ctgacatgca | gaatatattt | gcagttctct | tgagttagct | gatgaaaacc | caagagccag | 960 |
| cagggagtct | ggagggaaaac | aacagtgaca | agaacagtgg | gccacagggg | ccccgaagaa | 1020 |
| ctcccaacaat | gccacaggag | gaggcaggag | catgtctcc | tcacatttctt | ccaccagaga | 1080 |
| agaggcccccc | tgagcccccc | ggacctccac | cggcccccacc | tccacccctt | ctgggtgaag | 1140 |
| gcgatcttc | cagccccccc | caggagtga | acccaggccgt | gacagccccc | ttgctgcaac | 1200 |
| ttttatccca | gcttgaagca | gaggcctctt | gccaccttgc | acatgagcac | caggccttga | 1260 |
| gaccaatgga | gtactccacc | cgacccctgc | caaacaggac | ttatggaaac | actgtatggc | 1320 |
| ctgaaacagg | gttcagtgc | attgacactg | atgaacgaaa | ctctgttcca | gccttgacag | 1380 |
| aatccttgg | ccagaccctg | gtgaagaaca | ggaccttctc | aggctctctg | agccacccctt | 1440 |
| gggagttccag | cagttaaccag | ggcacagggt | cagtgcagtt | tccaggggac | caggacccctt | 1500 |
| gttttgcctag | ggtccccc | gcgttacacc | cggtggctgg | gcaaccatc | ctgaaggctg | 1560 |
| agggaaagcg | caattctgt | gtacatgcag | agaccaaatt | gcaaaactat | ggggagctgg | 1620 |
| ggccaggaac | cactggggcc | agcagctcag | gagcaggcc | tcaactgggg | ggcccaactc | 1680 |
| agtcttctgc | ttatggaaaa | ctctatcggg | ggccatcacaag | agtcccacca | agagggggaa | 1740 |
| gagggaggg | agttccttac | taacccagag | acttcagtgt | cctgaaagat | tcctttccta | 1800 |
| tccatccctc | catccagttc | tctgaatctt | taatgaaatc | atttgcccaga | gcgaggtaat | 1860 |
| catctgcatt | tggctactgc | aaagctgtcc | gttgtattcc | ttgctcactt | gtctactagca | 1920 |
| ggcgcattac | gaaataatga | tgttggacc | agttccccc | ggatggggct | tagccagaac | 1980 |
| atttacttca | actttactt | agtagataca | agtagagata | atggagaga | tcattacatt | 2040 |
| gaaaagtaaa | tgttttata | gttcatttgc | tgcaactact | gatcggaaaga | gagaaagaac | 2100 |
| agtttctaga | ttgagatggc | tcaggagagg | ctctttgatt | ttttaagttt | ttgggtgggg | 2160 |
| ggttgtgtgt | ggtttcttcc | tttgaattt | taattttaggt | gttttgggtt | tttttccctt | 2220 |
| aaagagaata | gtgttccaaa | aatttgcgt | gctcttgc | ttttgtata | agggaaaacag | 2280 |
| agtggcttgg | ctgattttgaa | taaatgttcc | tttccctctcc | accatctcac | attttgcctt | 2340 |
| taagtgcaca | ctttttccccc | attgagatc | ttgaaacatac | ttttttcca | aataaattac | 2400 |
| tcatccctaa | agtttactcc | actttgcaca | aaagatacgcc | cttctccctg | cataaaagc | 2460 |
| aggttgtaga | acgtggcatt | cttggcaag | tagtagact | ttacccagtc | tctttcctt | 2520 |
| tttgcgtatg | tgtgtctct | ctctctcttt | ctctctctct | ctctctctct | ctctctctct | 2580 |
| gtctgtctcg | cttgctcgct | ctcgctgttt | ctctctcttt | gaggcatttg | tttggaaaaaa | 2640 |
| atcgttgcata | tgcccaagaa | cctggataa | ttctttactt | ttttgtaaat | aaaggaaagg | 2700 |
| aaattcagac | tcttacattt | ttctctgtaa | ctcttcaatt | ctaaaatgtt | ttgtttttta | 2760 |
| aaccatgtt | tgatggggaa | gttattttgt | aagtgtggac | agcttggaca | ttgctgctga | 2820 |
| gctgtgttta | gagatgtc | ctccatttct | agagggtctaa | taacagcatt | tagcatattt | 2880 |
| tttcacata | tattttat | tcaaaaaaaaa | aacaaaaacc | tttcaaaacag | agcattgtga | 2940 |
| tattgtcaaa | gagaaaaaca | aatcctgaag | atacatggaa | atgtaaacctt | gttttaggtt | 3000 |
| ggttattttc | tgaagataca | tcaatacctg | acctttttta | aaaaaaataat | tttaaaacag | 3060 |
| cataactgtg | ggaagaacacag | tattgacata | cccacatccc | agcatgttta | ccctgccagt | 3120 |
| tcttttaggg | attttccctc | caaagagatt | tggatttgg | tttggtaaaa | gggggttaaat | 3180 |
| tgtgttccca | ggcaagaact | ttgccttatac | ataaaacgga | aatggaaaaag | ggaaggggctg | 3240 |
| tcaggatggg | ataattttgg | aggcttctca | ttctggctt | tatttctatgt | tgagtaccag | 3300 |
| catataagat | gtttaaaaaa | cagatacatgc | tcatataatt | tatctgcaca | gacttagacc | 3360 |
| ttcagggaaac | ataggttaag | cccccttta | caaagaaaaaa | gtaaacatac | ttcagcatct | 3420 |
| tggagggtag | ttttcaaaaac | tcaagtttca | tgtttcaatg | ccaagtttctt | attttaaaaaa | 3480 |
| ataaaatcta | cttataagaa | aaaaaaaaaa | aaaaaa | | | 3515 |

<210> 11
<211> 1258
<212> DNA
<213> Homo sapiens

<400> 11

| | | | | | | |
|-------------|--------------|-------------|--------------|-------------|------------|------|
| tctacacagg | gctgaaggct | gggctgaccc | tggagacacg | tttgcagata | gcactagatg | 60 |
| tggtggaggg | aatcccgcttc | ctgcacagcc | agggacttgt | ccatcgtat | atcaaactga | 120 |
| aaaatgtgct | gctggataag | cagaaccgtg | ccaagatcac | tgacttagga | ttctgcaagc | 180 |
| cagaggccat | gatgtcaggc | agcattgtgg | ggacaccaat | ccatatggcc | cctgaacttt | 240 |
| tcacaggaa | gtacgataat | tccgtggatg | tctacgctt | ttggatttt | ttctggata | 300 |
| tctgctcagg | ctctgtcaag | ctccctgagg | catttgagag | gtgtgctagc | aaagaccatc | 360 |
| tctggAACAA | tgtgcggagg | ggggctcgcc | cagaacgtct | tcctgtgtt | gatgaggagt | 420 |
| gctggcagtt | gatggaaGCC | tgttggatg | gcgcacccctt | gaagaggcct | ctcttggca | 480 |
| ttgtccagcc | catgctccag | ggcatcatga | atcggctctg | caagtccaaat | tctgagcagc | 540 |
| caaacagagg | actatgtat | tctacttggaa | agcaaaagacc | tttcttcttc | actctctatg | 600 |
| tatttccttc | ccccctcacct | tttggccatg | gggagaattt | gacattttt | cactatagga | 660 |
| cacactccca | agggaactgg | tgcttgcgtt | gaaaatttggaa | accttcccag | gcaggatgt | 720 |
| ctcctggaca | gtgaagagtt | gaatgactga | gcattttcag | cagctcactg | aagcgccaag | 780 |
| ctatccctt | agcaaaaaaaag | tgtctcagat | gtgtaaaagc | tgaggaatgt | ggtggcttgg | 840 |
| cttcacaaat | gaaaaggagg | cagatgttac | cattgtctt | tcactgtata | tacttctaag | 900 |
| acagcaagcg | ggacactgtca | gtggcaatag | tgttaaaaaaa | tctcattttc | atgattttt | 960 |
| gctctagcta | ggaatagttt | agtcaaggact | cagaaaattt | gagcttagtt | ctgggattat | 1020 |
| gaaaacatgg | ggcacaaaaac | aataacttgt | ggatgtctgg | ttcctgtgt | ctgcagccag | 1080 |
| gtatctcagg | ttgcaatggt | cagaagtccc | agtgaggggag | ctagaaacag | agctatctgt | 1140 |
| tcctcatagt | gccagtgtgt | ttacatttat | aggaccacat | atccctgggtt | tctggggag | 1200 |
| ggtttcaactg | ttacatcacc | aagaggccct | gaaaagagga | aaaaaaaaaa | aaaaaaaaaa | 1258 |

<210> 12

<211> 3240

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (127)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (136)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (3139)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (3187)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (3210)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (3226)

<223> n equals a,t,g, or c

<400> 12

| | | | | | | |
|--------------|------------|-------------|------------|------------|------------|-----|
| agcttggcac | gacagttttc | ccgacttgaa | aaccggccag | taagcgcaac | gcawttaatg | 60 |
| tgagttagct | cactcattag | gcaccccaagg | cttacactt | tatgcttcgg | gctcgatgt | 120 |
| tgtgtgnaat | tgtgancgga | taccawtttc | acacaggaac | cagctatgac | catgattacg | 180 |
| ccaagctcta | atacgactca | ctatagggaa | agctggatcg | cctgcaggtt | ccggccggaa | 240 |
| attcccccgggt | cgacccacgc | gtccgggttc | ctggttataa | tagttgctgg | gaccttattc | 300 |

| | | | | | | |
|-------------|-------------|-------------|------------|-------------|-------------|------|
| tattctgaat | ccccagcccc | taaagaaggc | ttggtactga | cgtgtacagc | gtagttaccg | 360 |
| agtgactttg | gggaaggcag | gaagagtgt | gaaaggacag | gaggaaggaa | gggaggggcc | 420 |
| gtggctccga | gctgcctaga | aaggcgctcc | agggagccag | ggcacccgt | gcctgggtgg | 480 |
| tggcagctgg | agccacgtcg | gagggggaaag | tgtcccgac | ttctctgcag | gcatcacaga | 540 |
| cctgaggcag | tggcctccgg | agggcactgg | acagaaacag | ccatccaat | ggctgagtgg | 600 |
| agggacccctg | ctcaagtgc | gtcgagtt | cggggtttc | cctcaggtag | ggatcggg | 660 |
| gccttgcgc | cgccagccac | gtgtggcg | cggtacagtc | agcagagtg | agggtcg | 720 |
| caccagaaaa | ggggcgccag | gggaactccc | gccccctcg | cgttgc | aaaacttc | 780 |
| gggcaggagg | cggtcg | aaagaagg | gaagagcg | cttttggaa | ctgtgcacgg | 840 |
| gacagattgg | acgcacacccc | ctcgggaggc | gcgaaggcat | ggaaaattt | aagcatatta | 900 |
| tcacccttgg | ccaggtcata | cacaacgt | gtgaagagat | gaaatactgc | aagaaacagt | 960 |
| gcccggccct | ggggcacccgc | gtcctcg | tgatccaag | tctggagat | ctccaggacc | 1020 |
| aaggaagag | gagcgtgccc | tctgagaat | taaccacag | catgaa | ccgc | 1080 |
| ccctggagga | ggctaatggg | gagatagaaa | agttcagca | tagatccaat | atctgcagg | 1140 |
| ttctaaccgc | aagccaggac | aaaatactct | tcaaggacgt | gaacaggaa | ctgagtgt | 1200 |
| tctggaaagga | gctctcg | ttacttcagg | ttgagca | catgcctt | tcacccataa | 1260 |
| gccaaggagc | gtcctggca | caggaagatc | agcaggat | agacgaagac | aggcgagct | 1320 |
| tccagatgct | aagaagagat | aatggaaaaa | tagaagctt | actgagac | tttagaaatca | 1380 |
| acatgaaaaa | aatcaaggaa | actttgg | aggat | accaaaaatgc | atgcaggaga | 1440 |
| tcccgcaga | gcaaatcaag | gagatcaaga | aggacg | ttcaggat | ccgtggattc | 1500 |
| tgctaaagg | aaatgaagtc | agcacactt | ataaaagg | ataccacaga | gctccagtgg | 1560 |
| ccataaaagt | attcaaaaaaa | ctccagg | gcagcatt | aatagtgg | cagacttca | 1620 |
| ataaggagat | caaaaaccatg | aagaaattc | aatctccaa | catc | tgctg | 1680 |
| tytgcattga | tgaacagtg | actccgc | attctccat | tgtcatgg | tactgt | 1740 |
| tcgggaccct | gagggagctg | ttggatagg | aaaaagac | cacacttgc | aagcgc | 1800 |
| tcctagtc | ggggcagcc | cgaggcctt | accggct | ccatc | gcac | 1860 |
| tccacgaaa | aatcaga | tcaacttcc | tggtaact | aggctac | gtgaag | 1920 |
| caggattga | gttggggaaa | acacagactt | ccatg | gggactac | agaaaaa | 1980 |
| cagacagat | caatctaca | gcatatct | caccc | actgg | agat | 2040 |
| aatatgatgt | aaagtctgaa | atatacag | ttggat | cctctgg | atcgcc | 2100 |
| gagatatccc | gttcaaggc | tgtaattt | agaagat | ccg | caag | 2160 |
| ggcagcagga | gccactgggt | gaagactg | cttca | gggagatc | attgtat | 2220 |
| gcccggccca | tgtccctct | gtcgg | ctgtt | aatctt | aaactcttca | 2280 |
| ccttttctaa | gtatgttac | aaaatctaa | ccaagg | tctgg | gaca | 2340 |
| aggcacaac | tggacat | tctctct | atcc | ttt | aatgtgg | 2400 |
| aaggagtgg | cacgctt | tgttaca | at | gggtt | tat | 2460 |
| cccactccaa | atgatattt | caaaaacata | cctc | ttt | atgtgg | 2520 |
| tcaaatgtat | ctttctgggt | atcc | tgg | caatg | ctc | 2580 |
| ccataaatga | agtctt | cccaccat | gaat | ctgg | actt | 2640 |
| atcaatagaa | tgtggagaa | gtactgt | gcc | ctt | gtt | 2700 |
| tttaaatgt | ctgtggaa | tttccc | cat | ttt | gtt | 2760 |
| atgagagacc | acatgaagca | aaaacat | ctt | ttt | atgtt | 2820 |
| acatggcagc | taacacatg | atgagg | tca | ttt | atgtt | 2880 |
| ccagccaaa | ttgcccattc | acacaat | gag | ttt | atgtt | 2940 |
| aaaaaaaaaa | agggcgcccg | ctct | ggg | ttt | atgtt | 3000 |
| catagctt | ctata | acctaaat | ttt | ttt | atgtt | 3060 |
| gactggaaa | accctggcgt | taccaact | aat | ttt | atgtt | 3120 |
| agctggcgt | atagcgaana | ttt | ttt | ttt | atgtt | 3180 |
| aatggcnaat | gggacgcccgc | cctgt | ttt | ttt | atgtt | 3240 |

<210> 13
<211> 793
<212> DNA
<213> Homo sapiens

| | | | | | | |
|------------|------------|------------|----------|-----------|-----------|-----|
| <400> 13 | 60 | | | | | |
| ggctcaagca | gaaatcaa | tacagaact | gaatagt | tttcagg | gtaaaaagg | 120 |
| cttggagtt | gaacctgg | acgtaaagg | tcttctcg | cgtgctact | catataaa | 180 |
| tcaaaacaag | ctccgggaa | ctacagaaga | tttggat | gtactgat | ttgagcct | 240 |
| taatgattt | gccaagaaaa | ccttgtc | gggtggaa | gatctgaaa | attctgaa | 300 |
| tgcattcg | actcaaacc | aaggaaaag | gatgg | tggat | cggaaat | 360 |
| agatgaagaa | ggaaaaagg | gaagaaa | tgaatgg | gggtgg | gatggat | 420 |
| attttctt | tttaggtt | tttggat | tttggat | tttggat | tttggat | 480 |
| ttctattcg | acagaaattt | gtatctat | agtttctt | gtttgc | tttggat | 540 |
| aagccagg | tttggat | cagcttgg | tttggat | tttggat | tttggat | |

| | |
|---|-----|
| ccttcttaac ctgtcacctt tttctagtgc tcagttaat tcctggcat caccttaaa | 600 |
| tgtaaatggc tgggtttatt tgacacagac cattgccag ttaactttaa atatctct | 660 |
| ttgataattg ttgttatat atctactgta ttgcaaacaa atgtgttggt ttcaaaaacag | 720 |
| gtatggtag ccccatttat atgcaaaaa tgaacgtatt actagaaaaaaa aaaaaaaaaa | 780 |
| aaaaaaaaaaa aaa | 793 |

<210> 14
<211> 302
<212> PRT
<213> Homo sapiens

| | |
|---|--|
| <400> 14 | |
| Met Asp Glu Gln Ser Gln Gly Met Gln Gly Pro Pro Val Pro Gln Phe | |
| 1 5 10 15 | |
| Gln Pro Gln Lys Ala Leu Arg Pro Asp Met Gly Tyr Asn Thr Leu Ala | |
| 20 25 30 | |
| Asn Phe Arg Ile Glu Lys Lys Ile Gly Arg Gly Gln Phe Ser Glu Val | |
| 35 40 45 | |
| Tyr Arg Ala Ala Cys Leu Leu Asp Gly Val Pro Val Ala Leu Lys Lys | |
| 50 55 60 | |
| Val Gln Ile Phe Asp Leu Met Asp Ala Lys Ala Arg Ala Asp Cys Ile | |
| 65 70 75 80 | |
| Lys Glu Ile Asp Leu Leu Lys Gln Leu Asn His Pro Asn Val Ile Lys | |
| 85 90 95 | |
| Tyr Tyr Ala Ser Phe Ile Glu Asp Asn Glu Leu Asn Ile Val Leu Glu | |
| 100 105 110 | |
| Leu Ala Asp Ala Gly Asp Leu Ser Arg Met Ile Lys His Phe Lys Lys | |
| 115 120 125 | |
| Gln Lys Arg Leu Ile Pro Glu Arg Thr Val Trp Lys Tyr Phe Val Gln | |
| 130 135 140 | |
| Leu Cys Ser Ala Leu Glu His Met His Ser Arg Arg Val Met His Arg | |
| 145 150 155 160 | |
| Asp Ile Lys Pro Ala Asn Val Phe Ile Thr Ala Thr Gly Val Val Lys | |
| 165 170 175 | |
| Leu Gly Asp Leu Gly Leu Gly Arg Phe Phe Ser Ser Lys Thr Thr Ala | |
| 180 185 190 | |
| Ala His Ser Leu Val Gly Thr Pro Tyr Tyr Met Ser Pro Glu Arg Ile | |
| 195 200 205 | |
| His Glu Asn Gly Tyr Asn Phe Lys Ser Asp Ile Trp Ser Leu Gly Cys | |
| 210 215 220 | |
| Leu Leu Tyr Glu Met Ala Ala Leu Gln Ser Pro Phe Tyr Gly Asp Lys | |
| 225 230 235 240 | |
| Met Asn Leu Tyr Ser Leu Cys Lys Lys Ile Glu Gln Cys Asp Tyr Pro | |
| 245 250 255 | |
| Pro Leu Pro Ser Asp His Tyr Ser Glu Glu Leu Arg Gln Leu Val Asn | |
| 260 265 270 | |
| Met Cys Ile Asn Pro Asp Pro Glu Lys Arg Pro Asp Val Thr Tyr Val | |
| 275 280 285 | |

Tyr Asp Val Ala Lys Arg Met His Ala Cys Thr Ala Ser Ser
290 295 300

<210> 15
<211> 800
<212> PRT
<213> Homo sapiens

<400> 15
Met Ser Ser Leu Gly Ala Ser Phe Val Gln Ile Lys Phe Asp Asp Leu
1 5 10 15

Gln Phe Phe Glu Asn Cys Gly Gly Ser Phe Gly Ser Val Tyr Arg
20 25 30

Ala Lys Trp Ile Ser Gln Asp Lys Glu Val Ala Val Lys Lys Leu Leu
35 40 45

Lys Ile Glu Lys Glu Ala Glu Ile Leu Ser Val Leu Ser His Arg Asn
50 55 60

Ile Ile Gln Phe Tyr Gly Val Ile Leu Glu Pro Pro Asn Tyr Gly Ile
65 70 75 80

Val Thr Glu Tyr Ala Ser Leu Gly Ser Leu Tyr Asp Tyr Ile Asn Ser
85 90 95

Asn Arg Ser Glu Glu Met Asp Met Asp His Ile Met Thr Trp Ala Thr
100 105 110

Asp Val Ala Lys Gly Met His Tyr Leu His Met Glu Ala Pro Val Lys
115 120 125

Val Ile His Arg Asp Leu Lys Ser Arg Asn Val Val Ile Ala Ala Asp
130 135 140

Gly Val Leu Lys Ile Cys Asp Phe Gly Ala Ser Arg Phe His Asn His
145 150 155 160

Thr Thr His Met Ser Leu Val Gly Thr Phe Pro Trp Met Ala Pro Glu
165 170 175

Val Ile Gln Ser Leu Pro Val Ser Glu Thr Cys Asp Thr Tyr Ser Tyr
180 185 190

Gly Val Val Leu Trp Glu Met Leu Thr Arg Glu Val Pro Phe Lys Gly
195 200 205

Leu Glu Gly Leu Gln Val Ala Trp Leu Val Val Glu Lys Asn Glu Arg
210 215 220

Leu Thr Ile Pro Ser Ser Cys Pro Arg Ser Phe Ala Glu Leu Leu His
225 230 235 240

Gln Cys Trp Glu Ala Asp Ala Lys Lys Arg Pro Ser Phe Lys Gln Ile
245 250 255

Ile Ser Ile Leu Glu Ser Met Ser Asn Asp Thr Ser Leu Pro Asp Lys
260 265 270

Cys Asn Ser Phe Leu His Asn Lys Ala Glu Trp Arg Cys Glu Ile Glu
275 280 285

Ala Thr Leu Glu Arg Leu Lys Lys Leu Glu Arg Asp Leu Ser Phe Lys

| 290 | 295 | 300 |
|-----|-----|-----|
| Glu | Gln | Glu |
| Lys | Glu | Leu |
| 305 | 310 | |
| Glu | Arg | Glu |
| Arg | Arg | Arg |
| 315 | 315 | |
| Leu | Lys | Met |
| | | Trp |
| | | Glu |
| | | Gln |
| 320 | | |
| Lys | Leu | Thr |
| Glu | Gln | Ser |
| Asn | Thr | Pro |
| 325 | 325 | Leu |
| Leu | Pro | Pro |
| | Ser | Phe |
| | | Glu |
| | | Ile |
| 330 | 330 | |
| 335 | | |
| Gly | Ala | Trp |
| Thr | Glu | Asp |
| Asp | Val | Tyr |
| 340 | 345 | Cys |
| Trp | Val | Trp |
| Val | Gln | Gln |
| | Leu | Leu |
| | | Val |
| 345 | 350 | |
| Arg | Lys | Gly |
| Gly | Asp | Ser |
| Ser | Ala | Glu |
| 355 | 360 | Met |
| | | Ser |
| | | Val |
| | | Tyr |
| | | Ala |
| | | Ser |
| | | Leu |
| | | Phe |
| 360 | 365 | |
| Lys | Glu | Asn |
| Asn | Ile | Thr |
| | Gly | Lys |
| | Arg | Leu |
| 370 | 375 | Leu |
| | | Leu |
| | | Glu |
| | | Glu |
| 375 | 380 | |
| Asp | Leu | Lys |
| Lys | Asp | Met |
| | Gly | Ile |
| | Val | Ser |
| 385 | 390 | Lys |
| | | Gly |
| | | His |
| | | Ile |
| | | Ile |
| | | His |
| 390 | 395 | Phe |
| | | |
| Lys | Ser | Ala |
| Ile | Glu | Lys |
| Asn | Leu | Thr |
| | His | Asp |
| | Tyr | Tyr |
| 405 | 410 | Ile |
| | | Asn |
| | | Leu |
| | | Phe |
| | | His |
| 410 | 415 | |
| Phe | Pro | Pro |
| Leu | Ile | Lys |
| Asp | Ser | Gly |
| | | Gly |
| | | Glu |
| 420 | 425 | Pro |
| | | Glu |
| | | Asn |
| | | Glu |
| 425 | 430 | |
| Glu | Lys | Ile |
| Val | Asn | Leu |
| | | Glu |
| | | Leu |
| | | Val |
| 435 | 440 | Phe |
| | | Gly |
| | | Phe |
| | | His |
| 440 | 445 | Leu |
| | | Lys |
| | | Pro |
| 445 | | |
| Gly | Thr | Gly |
| Pro | Gln | Asp |
| Cys | Lys | Trp |
| Lys | Met | Tyr |
| 450 | 455 | Met |
| | | Tyr |
| | | Glu |
| | | Met |
| 455 | 460 | Asp |
| | | |
| Gly | Asp | Glu |
| Ile | Ala | Ile |
| Asn | Thr | Tyr |
| | | Ile |
| 465 | 470 | Lys |
| | | Asp |
| | | Val |
| | | Thr |
| | | Phe |
| | | Asn |
| | | Thr |
| 470 | 475 | |
| 480 | | |
| Asn | Leu | Pro |
| Pro | Asp | Ala |
| | | Glu |
| | | Ile |
| | | Leu |
| | | Lys |
| 485 | 490 | Met |
| | | Thr |
| | | Lys |
| | | Pro |
| | | Pro |
| | | Phe |
| | | Val |
| 490 | 495 | |
| Met | Glu | Lys |
| Trp | Ile | Val |
| | Gly | Ile |
| | Ala | Lys |
| 500 | 505 | Ser |
| | | Gln |
| | | Thr |
| | | Val |
| | | Glu |
| 505 | 510 | Cys |
| | | |
| Thr | Val | Thr |
| Tyr | Glu | Ser |
| Asp | Val | Arg |
| | | Thr |
| 515 | 520 | Pro |
| | | Lys |
| | | Ser |
| | | Thr |
| | | Lys |
| 520 | 525 | His |
| | | |
| Val | His | Leu |
| Ile | Gln | Trp |
| | | Ser |
| | | Arg |
| 530 | 535 | Thr |
| | | Lys |
| | | Pro |
| | | Gln |
| 535 | 540 | Asp |
| | | Glu |
| | | Val |
| | | Lys |
| 540 | | |
| Ala | Val | Gln |
| Leu | Ala | Ile |
| | Gln | Thr |
| | | Leu |
| | | Phe |
| 545 | 550 | Thr |
| | | Asn |
| | | Ser |
| | | Asp |
| | | Gly |
| 550 | 555 | Asn |
| | | |
| Pro | Gly | Ser |
| Arg | Ser | Asp |
| Ser | Ser | Ser |
| 565 | 570 | Ala |
| | | Asp |
| | | Cys |
| | | Gln |
| | | Trp |
| | | Leu |
| | | Asp |
| 570 | 575 | Thr |
| | | |
| Leu | Arg | Met |
| Arg | Gln | Ile |
| | | Ala |
| | | Ser |
| 580 | 585 | Asn |
| | | Thr |
| | | Ser |
| | | Leu |
| | | Gln |
| 585 | 590 | Arg |
| | | Ser |
| | | Gln |
| 590 | | |
| Ser | Asn | Pro |
| | | Ile |
| | | Leu |
| | | Gly |
| | | Ser |
| | | Pro |
| | | Phe |
| | | Phé |
| | | Ser |
| | | His |
| | | Phe |
| 595 | 600 | Asp |
| | | Gly |
| | | Gln |
| 600 | 605 | |
| Asp | Ser | Tyr |
| | | Ala |
| | | Ala |
| | | Val |
| 610 | 615 | Arg |
| | | Arg |
| | | Pro |
| | | Gln |
| | | Val |
| | | Pro |
| | | Ile |
| 615 | 620 | Lys |
| | | Tyr |
| 620 | | |
| Gln | Gln | Ile |
| Ile | Thr | Pro |
| | | Val |
| | | Asn |
| 625 | 630 | Gln |
| | | Ser |
| | | Arg |
| | | Ser |
| | | Ser |
| | | Pro |
| | | Thr |
| 630 | 635 | Gln |
| | | |
| Tyr | Gly | Leu |
| | | Thr |
| | | Lys |
| | | Asn |
| | | Phe |
| | | Ser |
| | | Ser |
| | | Leu |
| | | His |
| | | Leu |
| | | Asn |
| | | Ser |
| | | Arg |
| | | Asp |

| 645 | 650 | 655 |
|--|-----|-----|
| Ser Gly Phe Ser Ser Gly Asn Thr Asp Thr Ser Ser Glu Arg Gly Arg 660 | 665 | 670 |
| Tyr Ser Asp Arg Ser Arg Asn Lys Tyr Gly Arg Gly Ser Ile Ser Leu 675 | 680 | 685 |
| Asn Ser Ser Pro Arg Gly Arg Tyr Ser Gly Lys Ser Gln His Ser Thr 690 | 695 | 700 |
| Pro Ser Arg Gly Arg Tyr Pro Gly Lys Phe Tyr Arg Val Ser Gln Ser 705 | 710 | 715 |
| Ala Leu Asn Pro His Gln Ser Pro Asp Phe Lys Arg Ser Pro Arg Asp 725 | 730 | 735 |
| Leu His Gln Pro Asn Thr Ile Pro Gly Met Pro Leu His Pro Glu Thr 740 | 745 | 750 |
| Asp Ser Arg Ala Ser Glu Glu Asp Ser Lys Val Ser Glu Gly Gly Trp 755 | 760 | 765 |
| Thr Lys Val Glu Tyr Arg Lys Lys Pro His Arg Pro Ser Pro Ala Lys 770 | 775 | 780 |
| Thr Asn Lys Glu Arg Ala Arg Gly Asp His Arg Gly Trp Arg Asn Phe 785 | 790 | 795 |
| | | 800 |

<210> 16
<211> 416
<212> PRT
<213> Homo sapiens

| | | |
|--|-----|-----|
| <400> 16 | | |
| Met Ala His Ser Pro Val Ala Val Gln Val Pro Gly Met Gln Asn Asn 1 | 5 | 10 |
| | | 15 |
| Ile Ala Asp Pro Glu Glu Leu Phe Thr Lys Leu Glu Arg Ile Gly Lys 20 | 25 | 30 |
| Gly Ser Phe Gly Glu Val Phe Lys Gly Ile Asp Asn Arg Thr Gln Gln 35 | 40 | 45 |
| Val Val Ala Ile Lys Ile Ile Asp Leu Glu Ala Glu Asp Glu Ile 50 | 55 | 60 |
| Glu Asp Ile Gln Gln Glu Ile Thr Val Leu Ser Gln Cys Asp Ser Ser 65 | 70 | 75 |
| | | 80 |
| Tyr Val Thr Lys Tyr Tyr Gly Ser Tyr Leu Lys Gly Ser Lys Leu Trp 85 | 90 | 95 |
| Ile Ile Met Glu Tyr Leu Gly Gly Ser Ala Leu Asp Leu Leu Arg 100 | 105 | 110 |
| Ala Gly Pro Phe Asp Glu Phe Gln Ile Ala Thr Met Leu Lys Glu Ile 115 | 120 | 125 |
| Lys Lys Gly Leu Asp Tyr Leu His Ser Glu Lys Lys Ile His Arg Asp 130 | 135 | 140 |

Ile Lys Ala Ala Asn Val Leu Leu Ser Glu Gln Gly Asp Val Lys Leu
 145 150 155 160

Ala Asp Phe Gly Val Ala Gly Gln Leu Thr Asp Thr Gln Ile Lys Arg
 165 170 175

Asn Thr Phe Val Gly Thr Pro Phe Trp Met Ala Pro Glu Val Ile Gln
 180 185 190

Gln Ser Ala Tyr Asp Ser Lys Ala Asp Ile Trp Ser Leu Gly Ile Thr
 195 200 205

Ala Ile Glu Leu Ala Lys Gly Glu Pro Pro Asn Ser Asp Met His Pro
 210 215 220

Met Arg Val Leu Phe Leu Ile Pro Lys Asn Asn Pro Pro Thr Leu Val
 225 230 235 240

Gly Asp Phe Thr Lys Ser Phe Lys Glu Phe Ile Asp Ala Cys Leu Asn
 245 250 255

Lys Asp Pro Ser Phe Arg Pro Thr Ala Lys Glu Leu Leu Lys His Lys
 260 265 270

Phe Ile Val Lys Asn Ser Lys Lys Thr Ser Tyr Leu Thr Glu Leu Ile
 275 280 285

Asp Arg Phe Lys Arg Trp Lys Ala Glu Gly His Ser Asp Asp Glu Ser
 290 295 300

Asp Ser Glu Gly Ser Asp Ser Glu Ser Thr Ser Arg Glu Asn Asn Thr
 305 310 315 320

His Pro Glu Trp Ser Phe Thr Thr Val Arg Lys Lys Pro Asp Pro Lys
 325 330 335

Lys Val Gln Asn Gly Ala Glu Gln Asp Leu Val Gln Thr Leu Ser Cys
 340 345 350

Leu Ser Met Ile Ile Thr Pro Ala Phe Ala Glu Leu Lys Gln Gln Asp
 355 360 365

Glu Asn Asn Ala Ser Arg Asn Gln Ala Ile Glu Glu Leu Glu Lys Ser
 370 375 380

Ile Ala Val Ala Glu Ala Ala Cys Pro Gly Ile Thr Asp Lys Met Val
 385 390 395 400

Lys Lys Leu Ile Glu Lys Phe Gln Lys Cys Ser Ala Asp Glu Ser Pro
 405 410 415

<210> 17

<211> 534

<212> PRT

<213> Homo sapiens

<400> 17

Met His Ile Arg Arg Glu Ile Glu Ile Met Ser Ser Leu Asn His Pro
 1 5 10 15His Ile Ile Ala Ile His Glu Val Phe Glu Asn Ser Ser Lys Ile Val
 20 25 30

Ile Val Met Glu Tyr Ala Ser Arg Gly Asp Leu Tyr Asp Tyr Ile Ser
35 40 45

Glu Arg Gln Gln Leu Ser Glu Arg Glu Ala Arg His Phe Phe Arg Gln
50 55 60

Ile Val Ser Ala Val His Tyr Cys His Gln Asn Arg Val Val His Arg
65 70 75 80

Asp Leu Lys Leu Glu Asn Ile Leu Leu Asp Ala Asn Gly Asn Ile Lys
85 90 95

Ile Ala Asp Phe Gly Leu Ser Asn Leu Tyr His Gln Gly Lys Phe Leu
100 105 110

Gln Thr Phe Cys Gly Ser Pro Leu Tyr Ala Ser Pro Glu Ile Val Asn
115 120 125

Gly Lys Pro Tyr Thr Gly Pro Glu Val Asp Ser Trp Ser Leu Gly Val
130 135 140

Leu Leu Tyr Ile Leu Val His Gly Thr Met Pro Phe Asp Gly His Asp
145 150 155 160

His Lys Ile Leu Val Lys Gln Ile Ser Asn Gly Ala Tyr Arg Glu Pro
165 170 175

Pro Lys Pro Ser Asp Ala Cys Gly Leu Ile Arg Trp Leu Leu Met Val
180 185 190

Asn Pro Thr Arg Arg Ala Thr Leu Glu Asp Val Ala Ser His Trp Trp
195 200 205

Val Asn Trp Gly Tyr Ala Thr Arg Val Gly Glu Gln Glu Ala Pro His
210 215 220

Glu Gly Gly His Pro Gly Ser Asp Ser Ala Arg Ala Ser Met Ala Asp
225 230 235 240

Trp Leu Arg Arg Ser Ser Arg Pro Leu Leu Glu Asn Gly Ala Lys Val
245 250 255

Cys Ser Phe Phe Lys Gln His Ala Pro Gly Gly Ser Thr Thr Pro
260 265 270

Gly Leu Glu Arg Gln His Ser Leu Lys Lys Ser Arg Lys Glu Asn Asp
275 280 285

Met Ala Gln Ser Leu His Ser Asp Thr Ala Asp Asp Thr Ala His Arg
290 295 300

Pro Gly Lys Ser Asn Leu Lys Leu Pro Lys Gly Ile Leu Lys Lys Lys
305 310 315 320

Val Ser Ala Ser Ala Glu Gly Val Gln Glu Asp Pro Pro Glu Leu Ser
325 330 335

Pro Ile Pro Ala Ser Pro Gly Gln Ala Ala Pro Leu Leu Pro Lys Lys
340 345 350

Gly Ile Leu Lys Lys Pro Arg Gln Arg Glu Ser Gly Tyr Tyr Ser Ser
355 360 365

Pro Glu Pro Ser Glu Ser Gly Glu Leu Leu Asp Ala Gly Asp Val Phe
370 375 380

```

<210> 18
<211> 365
<212> PRT
<213> Homo sapiens

<400> 18
Met Leu Glu Pro Asp Pro Glu His Arg Pro Asp Ile Phe Gln Val Ser
 1           5           10          15
Tyr Phe Ala Phe Lys Phe Ala Lys Lys Asp Cys Pro Val Ser Asn Ile
 20          25          30
Asn Asn Ser Ser Ile Pro Ser Ala Leu Pro Glu Pro Met Thr Ala Ser
 35          40          45
Glu Ala Ala Ala Arg Lys Ser Gln Ile Lys Ala Arg Ile Thr Asp Thr
 50          55          60
Ile Gly Pro Thr Glu Thr Ser Ile Ala Pro Arg Gln Arg Pro Lys Ala
 65          70          75          80
Asn Ser Ala Thr Thr Ala Thr Pro Ser Val Leu Thr Ile Gln Ser Ser
 85          90          95
Ala Thr Pro Val Lys Val Leu Ala Pro Gly Glu Phe Gly Asn His Arg
100          105         110
Pro Lys Gly Ala Leu Arg Pro Gly Asn Gly Pro Glu Ile Leu Leu Gly
115          120          125
Gln Gly Pro Pro Gln Gln Pro Pro Gln Gln His Arg Val Leu Gln Gln
130          135          140
Leu Gln Gln Gly Asp Trp Arg Leu Gln Gln Leu His Leu Gln His Arg

```

| | | | |
|---|-----|-----|-----|
| 145 | 150 | 155 | 160 |
| His Pro His Gln | | | |
| 165 | 170 | 175 | |
| Gln His His His His | | | |
| 180 | 185 | 190 | |
| His His His His Leu Leu Gln Asp Ala Tyr Met Gln Gln Tyr Gln | | | |
| 195 | 200 | 205 | |
| His Ala Thr Gln Gln Gln Gln Met Leu Gln Gln Gln Phe Leu Met His | | | |
| 210 | 215 | 220 | |
| Ser Val Tyr Gln Pro Gln Pro Ser Ala Ser Gln Tyr Pro Thr Met Met | | | |
| 225 | 230 | 235 | 240 |
| Pro Gln Tyr Gln Gln Ala Phe Phe Gln Gln Gln Met Leu Ala Gln His | | | |
| 245 | 250 | 255 | |
| Gln Pro Ser Gln Gln Gln Ala Ser Pro Glu Tyr Leu Thr Ser Pro Gln | | | |
| 260 | 265 | 270 | |
| Glu Phe Ser Pro Ala Leu Val Ser Tyr Thr Ser Ser Leu Pro Ala Gln | | | |
| 275 | 280 | 285 | |
| Val Gly Thr Ile Met Asp Ser Ser Tyr Ser Ala Asn Arg Ser Val Ala | | | |
| 290 | 295 | 300 | |
| Asp Lys Glu Ala Ile Ala Asn Phe Thr Asn Gln Lys Asn Ile Ser Asn | | | |
| 305 | 310 | 315 | 320 |
| Pro Pro Asp Met Ser Gly Trp Asn Pro Phe Gly Glu Asp Asn Phe Ser | | | |
| 325 | 330 | 335 | |
| Lys Leu Thr Glu Glu Leu Leu Asp Arg Glu Phe Asp Leu Leu Arg | | | |
| 340 | 345 | 350 | |
| Ser Ser Lys Gly His Leu Lys Ala Tyr Phe Ala Ser Gln | | | |
| 355 | 360 | 365 | |
| <210> 19 | | | |
| <211> 429 | | | |
| <212> PRT | | | |
| <213> Homo sapiens | | | |
| <400> 19 | | | |
| Met Ala Leu Ala Leu Asp Tyr Leu Arg Gly Gln His Ile Ile His Arg | | | |
| 1 | 5 | 10 | 15 |
| Asp Val Lys Pro Asp Asn Ile Leu Leu Asp Glu Arg Gly His Ala His | | | |
| 20 | 25 | 30 | |
| Leu Thr Asp Phe Asn Ile Ala Thr Ile Ile Lys Asp Gly Glu Arg Ala | | | |
| 35 | 40 | 45 | |
| Thr Ala Leu Ala Gly Thr Lys Pro Tyr Met Ala Pro Glu Ile Phe His | | | |
| 50 | 55 | 60 | |
| Ser Phe Val Asn Gly Gly Thr Gly Tyr Ser Phe Glu Val Asp Trp Trp | | | |
| 65 | 70 | 75 | 80 |
| Ser Val Gly Val Met Ala Tyr Glu Leu Leu Arg Gly Trp Arg Pro Tyr | | | |
| 85 | 90 | 95 | |

Asp Ile His Ser Ser Asn Ala Val Glu Ser Leu Val Gln Leu Phe Ser
 100 105 110
 Thr Val Ser Val Gln Tyr Val Pro Thr Trp Ser Lys Glu Met Val Ala
 115 120 125
 Leu Leu Arg Lys Leu Leu Thr Val Asn Pro Glu His Arg Leu Ser Ser
 130 135 140
 Leu Gln Asp Val Gln Ala Ala Pro Ala Leu Ala Gly Val Leu Trp Asp
 145 150 155 160
 His Leu Ser Glu Lys Arg Val Glu Pro Gly Phe Val Pro Asn Lys Gly
 165 170 175
 Arg Leu His Cys Asp Pro Thr Phe Glu Leu Glu Met Ile Leu Glu
 180 185 190
 Ser Arg Pro Leu His Lys Lys Lys Arg Leu Ala Lys Asn Lys Ser
 195 200 205
 Arg Asp Asn Ser Arg Asp Ser Ser Gln Ser Glu Asn Asp Tyr Leu Gln
 210 215 220
 Asp Cys Leu Asp Ala Ile Gln Gln Asp Phe Val Ile Phe Asn Arg Glu
 225 230 235 240
 Lys Leu Lys Arg Ser Gln Asp Leu Pro Arg Glu Pro Leu Pro Pro Leu
 245 250 255
 Ser Pro Gly Met Leu Arg Ser Leu Trp Arg Thr Arg Arg Asn Ala Pro
 260 265 270
 Pro Cys Pro Cys Ala Ala Pro Phe Ala Pro Arg Pro Gly Ala Ala Arg
 275 280 285
 Pro Gly Cys Pro Trp Ser Ser Pro Leu Glu Leu Leu Trp Arg Leu Gly
 290 295 300
 Cys Gln Arg Glu Gly His Gly Pro Arg Pro Gly Ile His Val Pro Thr
 305 310 315 320
 Gln Pro Gly Trp Arg Cys Pro Gln Cys Pro Gly His Ile Ser His Leu
 325 330 335
 Arg Leu Val Val Val Gln Gly Thr Arg Gly Cys Gly Cys Arg Gly His
 340 345 350
 Leu Trp Arg Ala Phe Pro Val Gly Pro Arg Asp Pro Pro Arg Trp Arg
 355 360 365
 Lys Arg Cys Trp Ala Pro Ser Tyr Arg Ser Arg Gly Ala Gly Ala Met
 370 375 380
 Asp Gly Thr Gly Val Phe Val Pro Ala Gln Pro Gly Gly Cys Ala Arg
 385 390 395 400
 Pro Ser Ser Gln Gly Asp Pro Cys Ser Ala Gly Arg Gly Cys Pro Arg
 405 410 415
 Leu Gly Ser Val Leu Gly Gly Gln Gly His Gly Leu Gly
 420 425

<210> 20
 <211> 344

<212> PRT

<213> Homo sapiens

<400> 20

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Asp | Tyr | Ile | Pro | Gly | Gly | Asp | Met | Met | Ser | Leu | Leu | Ile | Arg | Met |
| 1 | | | | 5 | | | | | 10 | | | | 15 | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Val | Phe | Pro | Glu | His | Leu | Ala | Arg | Phe | Tyr | Ile | Ala | Glu | Leu | Thr |
| | | | | | 20 | | | 25 | | | | 30 | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Ala | Ile | Glu | Ser | Val | His | Lys | Met | Gly | Phe | Ile | His | Arg | Asp | Ile |
| | | | 35 | | | | 40 | | | | 45 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Pro | Asp | Asn | Ile | Leu | Ile | Asp | Leu | Asp | Gly | His | Ile | Lys | Leu | Thr |
| | | | | 50 | | 55 | | | | 60 | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Phe | Gly | Leu | Cys | Thr | Gly | Phe | Arg | Trp | Thr | His | Asn | Ser | Lys | Tyr |
| | 65 | | | | 70 | | | | 75 | | | 80 | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Gln | Lys | Gly | Ser | His | Val | Arg | Gln | Asp | Ser | Met | Glu | Pro | Ser | Asp |
| | | | | 85 | | | 90 | | | | 95 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Trp | Asp | Asp | Val | Ser | Asn | Cys | Arg | Cys | Gly | Asp | Arg | Leu | Lys | Thr |
| | | | | 100 | | | | 105 | | | | 110 | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Glu | Gln | Arg | Ala | Arg | Lys | Gln | His | Gln | Arg | Cys | Leu | Ala | His | Ser |
| | | | | 115 | | | 120 | | | | 125 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Val | Gly | Thr | Pro | Asn | Tyr | Ile | Ala | Pro | Glu | Val | Leu | Leu | Arg | Lys |
| | 130 | | | | | 135 | | | | 140 | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Tyr | Thr | Gln | Leu | Cys | Asp | Trp | Trp | Ser | Val | Gly | Val | Ile | Leu | Phe |
| | 145 | | | | 150 | | | | 155 | | | 160 | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Met | Leu | Val | Gly | Gln | Pro | Pro | Phe | Leu | Ala | Pro | Thr | Pro | Thr | Glu |
| | | | | 165 | | | 170 | | | | 175 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Gln | Leu | Lys | Val | Ile | Asn | Trp | Glu | Asn | Thr | Leu | His | Ile | Pro | Ala |
| | | | | 180 | | | | 185 | | | 190 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Val | Lys | Leu | Ser | Pro | Glu | Ala | Arg | Asp | Leu | Ile | Thr | Lys | Leu | Cys |
| | | | | 195 | | 200 | | | | | 205 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Ser | Ala | Asp | His | Arg | Leu | Gly | Arg | Asn | Gly | Ala | Asp | Asp | Leu | Lys |
| | | | | 210 | | 215 | | | | 220 | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | His | Pro | Phe | Phe | Ser | Ala | Ile | Asp | Phe | Ser | Ser | Asp | Ile | Arg | Lys |
| | 225 | | | | 230 | | | 235 | | | | 240 | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Pro | Ala | Pro | Tyr | Val | Pro | Thr | Ile | Ser | His | Pro | Met | Asp | Thr | Ser |
| | | | | 245 | | | | 250 | | | 255 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Phe | Asp | Pro | Val | Asp | Glu | Glu | Ser | Pro | Trp | Asn | Asp | Ala | Ser | Glu |
| | | | | 260 | | | 265 | | | | 270 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Ser | Thr | Lys | Ala | Trp | Asp | Thr | Leu | Thr | Ser | Pro | Asn | Asn | Lys | His |
| | | | | 275 | | | 280 | | | | 285 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Glu | His | Ala | Phe | Tyr | Glu | Phe | Thr | Phe | Arg | Arg | Phe | Phe | Asp | Asp |
| | 290 | | | | 295 | | | | 300 | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Gly | Tyr | Pro | Phe | Arg | Cys | Pro | Lys | Pro | Ser | Gly | Ala | Glu | Ala | Ser |
| | 305 | | | | 310 | | | | 315 | | | 320 | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Glu | Ser | Ser | Asp | Leu | Glu | Ser | Ser | Asp | Leu | Val | Asp | Gln | Thr |
| | | | | 325 | | | 330 | | | | 335 | | | | |

Glu Gly Cys Gln Pro Val Tyr Val
340

<210> 21
<211> 665
<212> PRT
<213> Homo sapiens

<400> 21
Met Thr Ser Ala Asn Lys Ala Ile Glu Leu Gln Leu Gln Val Lys Gln
1 5 10 15

Asn Ala Glu Glu Leu Gln Asp Phe Met Arg Asp Leu Glu Asn Trp Glu
20 25 30

Lys Asp Ile Lys Gln Lys Asp Met Glu Leu Arg Arg Gln Asn Gly Val
35 40 45

Pro Glu Glu Asn Leu Pro Pro Ile Arg Asn Gly Asn Phe Arg Lys Lys
50 55 60

Lys Lys Gly Lys Ala Lys Glu Ser Ser Lys Lys Thr Arg Glu Glu Asn
65 70 75 80

Thr Lys Asn Arg Ile Lys Ser Tyr Asp Tyr Glu Ala Trp Ala Lys Leu
85 90 95

Asp Val Asp Arg Ile Leu Asp Glu Leu Asp Lys Asp Ser Thr His
100 105 110

Glu Ser Leu Ser Gln Glu Ser Glu Ser Glu Glu Asp Gly Ile His Val
115 120 125

Asp Ser Gln Lys Ala Leu Val Leu Lys Glu Lys Gly Asn Lys Tyr Phe
130 135 140

Lys Gln Gly Lys Tyr Asp Glu Ala Ile Asp Cys Tyr Thr Lys Gly Met
145 150 155 160

Asp Ala Asp Pro Tyr Asn Pro Val Leu Pro Thr Asn Arg Ala Ser Ala
165 170 175

Tyr Phe Arg Leu Lys Phe Ala Val Ala Glu Ser Asp Cys Asn Leu
180 185 190

Ala Val Ala Leu Asn Arg Ser Tyr Thr Lys Ala Tyr Ser Arg Arg Gly
195 200 205

Ala Ala Arg Phe Ala Leu Gln Lys Leu Glu Glu Ala Lys Lys Asp Tyr
210 215 220

Glu Arg Val Leu Glu Leu Glu Pro Asn Asn Phe Glu Ala Thr Asn Glu
225 230 235 240

Leu Arg Lys Ile Ser Gln Ala Leu Ala Ser Lys Glu Asn Ser Tyr Pro
245 250 255

Lys Glu Ala Asp Ile Val Ile Lys Ser Thr Glu Gly Glu Arg Lys Gln
260 265 270

Ile Glu Ala Gln Gln Asn Lys Gln Gln Ala Ile Ser Glu Lys Asp Arg
275 280 285

Gly Asn Gly Phe Phe Lys Glu Gly Lys Tyr Glu Arg Ala Ile Glu Cys

| 290 | 295 | 300 | |
|---|---|-----------------|-----|
| Tyr | Thr Arg Gly Ile Ala Ala Asp Gly Ala Asn Ala | Leu Leu Pro Ala | |
| 305 | 310 | 315 | 320 |
| Asn Arg Ala Met Ala Tyr Leu Lys Ile Gln Lys Tyr Glu Glu Ala Glu | | | |
| 325 | 330 | 335 | |
| Lys Asp Cys Thr Gln Ala Ile Leu Leu Asp Gly Ser Tyr Ser Lys Ala | | | |
| 340 | 345 | 350 | |
| Phe Ala Arg Arg Gly Thr Ala Arg Thr Phe Leu Gly Lys Leu Asn Glu | | | |
| 355 | 360 | 365 | |
| Ala Lys Gln Asp Phe Glu Thr Val Leu Leu Leu Glu Pro Gly Asn Lys | | | |
| 370 | 375 | 380 | |
| Gln Ala Val Thr Glu Leu Ser Lys Ile Lys Lys Glu Leu Ile Glu Lys | | | |
| 385 | 390 | 395 | 400 |
| Gly His Trp Asp Asp Val Phe Leu Asp Ser Thr Gln Arg Gln Asn Val | | | |
| 405 | 410 | 415 | |
| Val Lys Pro Ile Asp Asn Pro Pro His Pro Gly Ser Thr Lys Pro Leu | | | |
| 420 | 425 | 430 | |
| Lys Lys Val Ile Ile Glu Glu Thr Gly Asn Leu Ile Gln Thr Ile Asp | | | |
| 435 | 440 | 445 | |
| Val Pro Asp Ser Thr Thr Ala Ala Ala Pro Glu Asn Asn Pro Ile Asn | | | |
| 450 | 455 | 460 | |
| Leu Ala Asn Val Ile Ala Ala Thr Gly Thr Thr Ser Lys Lys Asn Ser | | | |
| 465 | 470 | 475 | 480 |
| Ser Gln Asp Asp Leu Phe Pro Thr Ser Asp Thr Pro Arg Ala Lys Val | | | |
| 485 | 490 | 495 | |
| Leu Lys Ile Glu Glu Val Ser Asp Thr Ser Ser Leu Gln Pro Gln Ala | | | |
| 500 | 505 | 510 | |
| Ser Leu Lys Gln Asp Val Cys Gln Ser Tyr Ser Glu Lys Met Pro Ile | | | |
| 515 | 520 | 525 | |
| Glu Ile Glu Gln Lys Pro Ala Gln Phe Ala Thr Thr Val Leu Pro Pro | | | |
| 530 | 535 | 540 | |
| Ile Pro Ala Asn Ser Phe Gln Leu Glu Ser Asp Phe Arg Gln Leu Lys | | | |
| 545 | 550 | 555 | 560 |
| Ser Ser Pro Asp Met Leu Tyr Gln Tyr Leu Lys Gln Ile Glu Pro Ser | | | |
| 565 | 570 | 575 | |
| Leu Tyr Pro Lys Leu Phe Gln Lys Asn Leu Asp Pro Asp Val Phe Asn | | | |
| 580 | 585 | 590 | |
| Gln Ile Val Lys Ile Leu His Asp Phe Tyr Ile Glu Lys Glu Lys Pro | | | |
| 595 | 600 | 605 | |
| Leu Leu Ile Phe Glu Ile Leu Gln Arg Leu Ser Glu Leu Lys Arg Phe | | | |
| 610 | 615 | 620 | |
| Asp Met Ala Val Met Phe Met Ser Glu Thr Glu Lys Lys Ile Ala Arg | | | |
| 625 | 630 | 635 | 640 |
| Ala Leu Phe Asn His Ile Asp Lys Ser Gly Leu Lys Asp Ser Ser Val | | | |

645

650

655

Glu Glu Leu Lys Lys Arg Tyr Gly Gly
 660 665

<210> 22
 <211> 519
 <212> PRT
 <213> Homo sapiens

<400> 22
 Met Lys Pro Lys Lys Gln Tyr Arg Arg Arg Leu Arg Glu Glu Phe Ser
 1 5 10 15

Phe Ile Pro Ser Ala Ala Leu Asp Leu Leu Asp His Met Leu Thr Leu
 20 25 30

Asp Pro Ser Lys Arg Cys Thr Ala Glu Gln Thr Leu Gln Ser Asp Phe
 35 40 45

Leu Lys Asp Val Glu Leu Ser Lys Met Ala Pro Pro Asp Leu Pro His
 50 55 60

Trp Gln Asp Cys His Glu Leu Trp Ser Lys Lys Arg Arg Arg Gln Arg
 65 70 75 80

Gln Ser Gly Val Val Val Glu Glu Pro Pro Pro Ser Lys Thr Ser Arg
 85 90 95

Lys Glu Thr Thr Ser Gly Thr Ser Thr Glu Pro Val Lys Asn Ser Ser
 100 105 110

Pro Ala Pro Pro Gln Pro Ala Pro Gly Lys Val Glu Ser Gly Ala Gly
 115 120 125

Asp Ala Ile Gly Leu Ala Asp Ile Thr Gln Gln Leu Asn Gln Ser Glu
 130 135 140

Leu Ala Val Leu Leu Asn Leu Leu Gln Ser Gln Thr Asp Leu Ser Ile
 145 150 155 160

Pro Gln Met Ala Gln Leu Leu Asn Ile His Ser Asn Pro Glu Met Gln
 165 170 175

Gln Gln Leu Glu Ala Leu Asn Gln Ser Ile Ser Ala Leu Thr Glu Ala
 180 185 190

Thr Ser Gln Gln Gln Asp Ser Glu Thr Met Ala Pro Glu Glu Ser Leu
 195 200 205

Lys Glu Ala Pro Ser Ala Pro Val Ile Leu Pro Ser Ala Glu Gln Thr
 210 215 220

Thr Leu Glu Ala Ser Ser Thr Pro Ala Asp Met Gln Asn Ile Leu Ala
 225 230 235 240

Val Leu Leu Ser Gln Leu Met Lys Thr Gln Glu Pro Ala Gly Ser Leu
 245 250 255

Glu Glu Asn Asn Ser Asp Lys Asn Ser Gly Pro Gln Gly Pro Arg Arg
 260 265 270

Thr Pro Thr Met Pro Gln Glu Ala Ala Ala Cys Pro Pro His Ile
 275 280 285

Leu Pro Pro Glu Lys Arg Pro Pro Glu Pro Pro Gly Pro Pro Pro Pro
 290 295 300
 Pro Pro Pro Pro Leu Val Glu Gly Asp Leu Ser Ser Ala Pro Gln
 305 310 315 320
 Glu Leu Asn Pro Ala Val Thr Ala Ala Leu Leu Gln Leu Leu Ser Gln
 325 330 335
 Pro Glu Ala Glu Pro Pro Gly His Leu Pro His Glu His Gln Ala Leu
 340 345 350
 Arg Pro Met Glu Tyr Ser Thr Arg Pro Arg Pro Asn Arg Thr Tyr Gly
 355 360 365
 Asn Thr Asp Gly Pro Glu Thr Gly Phe Ser Ala Ile Asp Thr Asp Glu
 370 375 380
 Arg Asn Ser Gly Pro Ala Leu Thr Glu Ser Leu Val Gln Thr Leu Val
 385 390 395 400
 Lys Asn Arg Thr Phe Ser Gly Ser Leu Ser His Leu Gly Glu Ser Ser
 405 410 415
 Ser Tyr Gln Gly Thr Gly Ser Val Gln Phe Pro Gly Asp Gln Asp Leu
 420 425 430
 Arg Phe Ala Arg Val Pro Leu Ala Leu His Pro Val Val Gly Gln Pro
 435 440 445
 Phe Leu Lys Ala Glu Gly Ser Ser Asn Ser Val Val His Ala Glu Thr
 450 455 460
 Lys Leu Gln Asn Tyr Gly Glu Leu Gly Pro Gly Thr Thr Gly Ala Ser
 465 470 475 480
 Ser Ser Gly Ala Gly Leu His Trp Gly Gly Pro Thr Gln Ser Ser Ala
 485 490 495
 Tyr Gly Lys Leu Tyr Arg Gly Pro Thr Arg Val Pro Pro Arg Gly Gly
 500 505 510
 Arg Gly Arg Gly Val Pro Tyr
 515

<210> 23
 <211> 126
 <212> PRT
 <213> Homo sapiens

<400> 23
 Met Met Ser Gly Ser Ile Val Gly Thr Pro Ile His Met Ala Pro Glu
 1 5 10 15
 Leu Phe Thr Gly Lys Tyr Asp Asn Ser Val Asp Val Tyr Ala Phe Gly
 20 25 30
 Ile Leu Phe Trp Tyr Ile Cys Ser Gly Ser Val Lys Leu Pro Glu Ala
 35 40 45
 Phe Glu Arg Cys Ala Ser Lys Asp His Leu Trp Asn Asn Val Arg Arg
 50 55 60
 Gly Ala Arg Pro Glu Arg Leu Pro Val Phe Asp Glu Glu Cys Trp Gln
 65 70 75 80

Leu Met Glu Ala Cys Trp Asp Gly Asp Pro Leu Lys Arg Pro Leu Leu
85 90 95

Gly Ile Val Gln Pro Met Leu Gln Gly Ile Met Asn Arg Leu Cys Lys
100 105 110

Ser Asn Ser Glu Gln Pro Asn Arg Gly Leu Asp Asp Ser Thr
115 120 125

<210> 24

<211> 471

<212> PRT

<213> Homo sapiens

<400> 24

Met Glu Asn Leu Lys His Ile Ile Thr Leu Gly Gln Val Ile His Lys
1 5 10 15

Arg Cys Glu Glu Met Lys Tyr Cys Lys Lys Gln Cys Arg Arg Leu Gly
20 25 30

His Arg Val Leu Gly Leu Ile Lys Pro Leu Glu Met Leu Gln Asp Gln
35 40 45

Gly Lys Arg Ser Val Pro Ser Glu Lys Leu Thr Thr Ala Met Asn Arg
50 55 60

Phe Lys Ala Ala Leu Glu Glu Ala Asn Gly Glu Ile Glu Lys Phe Ser
65 70 75 80

Asn Arg Ser Asn Ile Cys Arg Phe Leu Thr Ala Ser Gln Asp Lys Ile
85 90 95

Leu Phe Lys Asp Val Asn Arg Lys Leu Ser Asp Val Trp Lys Glu Leu
100 105 110

Ser Leu Leu Leu Gln Val Glu Gln Arg Met Pro Val Ser Pro Ile Ser
115 120 125

Gln Gly Ala Ser Trp Ala Gln Glu Asp Gln Gln Asp Ala Asp Glu Asp
130 135 140

Arg Arg Ala Phe Gln Met Leu Arg Arg Asp Asn Glu Lys Ile Glu Ala
145 150 155 160

Ser Leu Arg Arg Leu Glu Ile Asn Met Lys Glu Ile Lys Glu Thr Leu
165 170 175

Arg Gln Tyr Leu Pro Pro Lys Cys Met Gln Glu Ile Pro Gln Glu Gln
180 185 190

Ile Lys Glu Ile Lys Lys Glu Gln Leu Ser Gly Ser Pro Trp Ile Leu
195 200 205

Leu Arg Glu Asn Glu Val Ser Thr Leu Tyr Lys Gly Glu Tyr His Arg
210 215 220

Ala Pro Val Ala Ile Lys Val Phe Lys Lys Leu Gln Ala Gly Ser Ile
225 230 235 240

Ala Ile Val Arg Gln Thr Phe Asn Lys Glu Ile Lys Thr Met Lys Lys
245 250 255

Phe Glu Ser Pro Asn Ile Leu Arg Ile Phe Gly Ile Cys Ile Asp Glu

| 260 | 265 | 270 |
|---|-----|-----|
| Thr Val Thr Pro Pro Gln Phe Ser Ile Val Met Glu Tyr Cys Glu Leu | | |
| 275 | 280 | 285 |
| Gly Thr Leu Arg Glu Leu Leu Asp Arg Glu Lys Asp Leu Thr Leu Gly | | |
| 290 | 295 | 300 |
| Lys Arg Met Val Leu Val Leu Gly Ala Ala Arg Gly Leu Tyr Arg Leu | | |
| 305 | 310 | 315 |
| His His Ser Glu Ala Pro Glu Leu His Gly Lys Ile Arg Ser Ser Asn | | |
| 325 | 330 | 335 |
| Phe Leu Val Thr Gln Gly Tyr Gln Val Lys Leu Ala Gly Phe Glu Leu | | |
| 340 | 345 | 350 |
| Arg Lys Thr Gln Thr Ser Met Ser Leu Gly Thr Thr Arg Glu Lys Thr | | |
| 355 | 360 | 365 |
| Asp Arg Val Lys Ser Thr Ala Tyr Leu Ser Pro Gln Glu Leu Glu Asp | | |
| 370 | 375 | 380 |
| Val Phe Tyr Gln Tyr Asp Val Lys Ser Glu Ile Tyr Ser Phe Gly Ile | | |
| 385 | 390 | 395 |
| Val Leu Trp Glu Ile Ala Thr Gly Asp Ile Pro Phe Gln Gly Cys Asn | | |
| 405 | 410 | 415 |
| Ser Glu Lys Ile Arg Lys Leu Val Ala Val Lys Arg Gln Gln Glu Pro | | |
| 420 | 425 | 430 |
| Leu Gly Glu Asp Cys Pro Ser Glu Leu Arg Glu Ile Ile Asp Glu Cys | | |
| 435 | 440 | 445 |
| Arg Ala His Asp Pro Ser Val Arg Pro Ser Val Asp Glu Ile Leu Lys | | |
| 450 | 455 | 460 |
| Lys Leu Ser Thr Phe Ser Lys | | |
| 465 | 470 | |

<210> 25
<211> 78
<212> PRT
<213> Homo sapiens

<400> 25
Met Val Ile Gln Glu Ile Glu Asn Ser Glu Asp Glu Glu Gly Lys Ser
1 5 10 15
Gly Arg Lys His Glu Asp Gly Gly Asp Lys Ser Lys Ile Phe Phe
20 25 30
Leu Phe Arg Leu Cys Lys Lys Leu Pro Phe Asn Met Met Ser Trp Leu
35 40 45
Asn Phe Ser Ile Arg Thr Glu Ile Arg Asn Leu Ser Val Phe Leu Ala
50 55 60
Leu Pro Cys Lys Phe Thr Ser Gln Phe Arg Ser Ser Phe Ser
65 70 75

<210> 26
<211> 13

<212> PRT
<213> Homo sapiens

<400> 26
Val Met His Arg Asp Ile Lys Pro Ala Asn Val Phe Ile
1 5 10

<210> 27
<211> 13
<212> PRT
<213> Homo sapiens

<400> 27
Val Ile His Arg Asp Leu Lys Ser Arg Asn Val Val Ile
1 5 10

<210> 28
<211> 13
<212> PRT
<213> Homo sapiens

<400> 28
Val Val His Arg Asp Leu Lys Leu Glu Asn Ile Leu Leu
1 5 10

<210> 29
<211> 13
<212> PRT
<213> Homo sapiens

<400> 29
Ile Ile His Arg Asp Val Lys Pro Asp Asn Ile Leu Leu
1 5 10

<210> 30
<211> 13
<212> PRT
<213> Homo sapiens

<400> 30
Phe Ile His Arg Asp Ile Lys Pro Asp Asn Ile Leu Ile
1 5 10

<210> 31
<211> 13
<212> PRT
<213> Homo sapiens

<400> 31
Leu Val His Arg Asp Ile Lys Leu Lys Asn Val Leu Leu
1 5 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/21736

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07K 14/705, 14/715; C12N 5/10, 15/12, 15/63, 15/64

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.1, 23.5, 24.3, 24.31; 435/69.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CAS ONLINE, MEDLINE, CAPLUS

search terms: TGF-beta receptor family, polynucleotide, protein

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---------------|---|------------------------------------|
| X --- A | WO 98/11234 A2 (INCYTE PHARMACEUTICALS, INC.) 19 March 1998 (19/03/98), see entire document, especially pages 33-39. | 1, 7-11, 14-16 ----- 2-6, 12 |

Further documents are listed in the continuation of Box C.

See patent family annex.

| | | |
|---|------|--|
| * Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "B" earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "g." | document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | | |
| "P" document published prior to the international filing date but later than the priority date claimed | | |

Date of the actual completion of the international search

03 OCTOBER 2000

Date of mailing of the international search report

21 NOV 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer
Dorothy Lawrence for
PREMA MERTZ

Telephone No. (703) 308-0196

BEST AVAILABLE COPY

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/21736

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-12, 14-16, SEQ ID NO:2, 14

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/21736

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/350; 536/23.1, 23.5, 24.3, 24.31; 435/69.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-12,14-16 drawn to a nucleic acid of SEQ ID NO:2 encoding a protein of SEQ ID NO:14, a vector, a host cell, a method of making the protein and the protein of SEQ ID NO:14.

Group II, claim 13, drawn to an antibody that binds the protein of SEQ ID NO:14.

Group III, claim 17, drawn to a method of treating a condition comprising administering the protein of SEQ ID NO:14.

Group IV, claim 18, drawn to a method of diagnosing a pathological condition using the polynucleotide encoding a protein of SEQ ID NO:14.

Group V, claim 19, drawn to a method of diagnosing a pathological condition by determining the amount of protein of SEQ ID NO:14.

Group VI, claims 20-21, drawn to a method of identifying a binding partner of the protein of SEQ ID NO:14.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, a nucleic acid encoding a protein of SEQ ID NO:14, a vector, a host cell, a method of making the protein of SEQ ID NO:14, and the protein of SEQ ID NO:14. Further pursuant to 37

C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

the polynucleotides set forth in SEQ ID NO:2-13 encoding the polypeptides set forth in SEQ ID NO:14-25.